Results of the international ring test 2018 and 2019
Final report and overall conclusions from the several validation studies

Validation of the Homing flight test in honeybee (Apis mellifera L.) after single exposure to sublethal doses of a test chemical

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1-INTRODUCTION

According to the Regulation (EC) No 1107/2009 (Annex II point 3.8.3), an active substance or a formulated plant protection product, shall only be approved if it is carefully evaluated following an appropriate risk assessment. Among several factors, this includes acute and chronic effects on honeybee colony survival and development, considering effects on honeybee larvae and **honeybee behaviour**. For the latter, no standardized method exists to evaluate sublethal effects on foraging behaviour of honeybees. Sublethal effects in individual worker bees may have the potential to affect functions at colony level and/or colony survival (Henry et al. 2012, 2015, Woodcock et al. 2017). Recent revision of plant protection products' risk assessment on bees recommended the use of a homing flight test to study the effect of sublethal doses of plant protection products on this trait of interest (EFSA, 2013).

The homing test proposes to assess effects of a single, oral exposure to sublethal doses of a chemical (technical grade active substance or a formulation) on the homing performance of forager bees. Thereby, feeding solutions are administered under controlled conditions and subsequently foragers are released in order to mimic field realistic homing conditions.
The method project was adopted by the OECD Working Group of National Coordinators of the Test Guidelines Programme (WNT) and is integrated in the work plan of OECD since 2016.

Background of the OECD ring test foundation is presented in the previous report ("Results of the international ring test 2016 and 2017") for the validation of a homing flight test design.
The test' endpoint is the determination of a No-Observed-Effect-Dose (NOED) on the homing success of foragers released at a distance of 1 km (+/-100 m) away from the experimental colony. This distance is within the range that foragers routinely cover during normal foraging flights (Steffan-Dewenter & Kuhn, 2003, Park & Nieh, 2017). Moreover, the proposed ring test aims to establish a validity criterion of the studies regarding the minimum- and acceptable homing-success-rate of untreated control bees.
The active substance thiamethoxam was used as a reference item in this ring test, since several studies have demonstrated that thiamethoxam can negatively affect the homing ability of foragers (Henry et al. 2012, 2014, 2015). For each trial (from 2015-2017) we tested three sublethal doses of the active substance (according to a geometric progression with a ratio of 3): 0.11 ng, 0.33 ng and 1 ng per bee. Tested doses range was changed in 2018 and 2019 to 0.33 ng, 1 ng and 1.5 ng per bee to take into account possible differences in sensitivity of the bees around the dose of 1 ng per bee. Similarly, a control solution (acetone 0.1 % in a 30 % w/v sucrose solution) was included. All labs used technical grade thiamethoxam originating from the same batch number (purity = 99%). For each test run, bees were exposed collectively (in 10 bees-cages) to one of the four feeding solutions.
Homing performance was measured (for 24 hours) by monitoring free-ranging foragers with radio-frequency identification (RFID) tagging technology. For each treatment-group, both, homing success rate and its corresponding duration were calculated from the automatically saved data. For the interpretation of obtained results, the variability and potential causing factors were discussed.

Methodological improvements were continuously achieved based on experimental observations. From 2016, one main improvement of the method consisted to use an alternative method to that of the Phacelia approach as described in the first report in 2015 ("Summary of results of the First international ring test 2015"). The alternative method is based on the use of a colored dye powder used to stain forager bees, which allows the identification of bees at the hive entrance which were released at a distance of 1 km (+/-100 m) from the test colony. Then, it can be ensured that foragers have at least one successful homing trip and thus advanced knowledge of the pathway back to the colony from the release site. This alternative greatly improved the test feasibility and was validated. The other main proposal was the addition of a feeding phase *ad libitum* before the tagged bees' release to facilitate the energy level of the bees. But this food supply could appear as a source of variability

of homing results in exposed bees due to the dilution of the remaining volume of sucrose from the exposure phase in the bee crop. As a result, this feeding phase was suppressed from 2018, but the protocol was adjusted for good maintenance and performance of the bees during the laboratory phase with **i**) a pre-exposure starvation duration fixed to 1h30; **ii**) an exposure starvation performed in dark conditions during 1h prolonged for a maximum of 30 min if needed; **iii**) a decrease of the post-exposure starvation period from 1 h to 40 min as for Henry et al. (2012).

Additionally, studies carried out in 2018 by two labs of the ring test, pointed out the need to focus as far as possible on pollen foragers instead of only nectar foragers. Indeed, pollen collectors are expected to have relatively low stomach content. This helps for a better consumption and homogeneous distribution of the sucrose solution by trophallaxis among the bees during the exposure phase, and this may prevent possible dilution that could occur when only nectar foragers with expected higher stomach content are collected. Consequently, increase in accuracy of the effects measured with the doses tested is expected. Focus on pollen collectors was adopted in 2019.

Then, the ring test trials were pursued and the corresponding results obtained during 2018 and 2019 are presented in this report. Overall considerations of the results over the five years of ring test are also included.

2-INFORMATION ON THE RING TEST GROUP

In total, ten laboratories participated in the ring tests of 2018 and 2019. Participants represented a wide range of stakeholder groups, including governmental institutions, contract laboratories and technical bee institutes.

Laboratory	**Responsible person(s)**
ITSAP-Institut de l'Abeille, **France** ***Project leader, organiser of the ring test***	Julie Fourrier
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Agroscope, Swiss Bee Research Centre **Switzerland**	Lukas Jeker Daniela Grossar Michael Eyer
Ibacon, Institut für Biologische Analytik und Consulting Gmbh, **Germany**	Martin Benz Stephan Schmitzer Tatsuya Sekine
The Fera (Science) Ltd, **United Kingdom**	Selwyn Wilkins Emma Wright
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LAVES Institute for Apidology Celle, **Germany**	Martina Janke Dorothee Lueken
TESTAPI, **France**	Hervé Giffard Olivier Mamet

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3-RING TEST SCHEDULE (2018 and 2019)

	2018	2019
Start of experimental phase	May	May
End of experimental phase	September	September
Evaluation of results	July to December	July to December
Results presentation to the ring test group	January 2019	January 2020

4-MATERIAL AND METHODS

4.1 Honeybees

Source of the colonies, treatments and health status: Chemical treatments (anti-varroa…) have been completed at least four weeks before the start of the experiment. Queen-right (queens with known history and not older than 2 years) and healthy colonies (as far as possible disease-free) were used for the experiments.

Hives characteristics: Each test hive was equipped with 10 to 12-frames. It has to be checked that bees correctly circulate through RFID readers to get in and out of the colony (no cluster of bees at the hive entrance) and that no trophallaxis between inside and outside bees occurs at the bottom of the hive. According to climatic conditions, hive volume can be increased by adding one to two supers and good thermoregulation during summer climatic conditions will be ensured.

In 2019, it was added that strong and active colonies with enough brood and food stock are used for the test.

Preparation of the colonies: The colonies used for the ring test were homogenous in terms of colony strength, food storage, amount of brood and experimental preparation. Hives with ten frames configuration comprised five to seven brood combs of all stages (eggs, uncapped larvae and pupae) and hives with twelve frames configuration contained six to eight brood combs. Each hive configurations contained two to three food combs and at least one empty frame. A colony inspection (routine apiarist visit) was performed for each experimental colony one to four days before the test start to prepare the colony and to verify health status. Good colony activity was checked by monitoring the foraging activity at the hive entrance.

Varroa load: During each apiarist visit, a sample of bees on brood frames (± 200-400 bees) was collected. All samples were sent to ITSAP Lab. Honeybees were washed with water and detergent (Dietemann et al. 2013) in order to count the phoretic mites (*Varroa destructor*) and establish the number of varroas per 100 honeybees (Lee et al. 2010). This counting is an indicator of the colony's Varroa load.

Installation of the colonies: the colonies used for the test had to be installed on the experimental site, at least one week before the start of the test, to allow acclimatisation and familiarisation with the environment by the honeybees. If all the colonies were placed on the experimental site at the same

time, they were separated spatially by few meters (± 10 meters) and placed in a staggered configuration to maximally avoid drift of labelled bees between the colonies.

4.2 RFID device

RFID (Radio Frequency Identification) device: The RFID technology (Streit *et al*. 2003; Decourtye *et al*. 2011) allows detection each time an RFID tagged bee passed through the reader (working distance of 3 mm). The principle depends on the emission of a radio signal from the reader which is received by the tag on the bee's thorax. The tag is not equipped with a power source (passive function) and it obtains its operating power from the reading process to emit a unique identification code. The reader automatically recognizes a virtually unlimited number of individual insects.

For the five ring test years, the used tags worked with 13.56 MHz frequency; Microsensys GmbH, Erfurt, Germany (2.0 x 1.7 x 0.5 mm).They weighed no more than 3 mg, equivalent to approximately 3 % of the weight of a worker bee. RFID tags were glued with dental cement (Temposil 2) on the dorsal side of the thorax of the bees. The used RFID system was MAJA system (Microsensys GmbH, Erfurt, Germany). It comprised of one Host (small computer with a Windows system) that recorded data of all forager passing's on a SD card. Four readers were placed at the entrance of the hive (parallel arrangement). Each reader spanned a tunnel of 14 × 21.5 mm (7 mm high) acting as an entrance to the colony. Readers were installed at the hive entrance thanks to an interface (in plastic or wood) between hive and readers (= mask). Then, the bees were able to enter the hive by passing through the 4 possible entrances formed by the readers (**Figure 1**, **Appendix 1**).

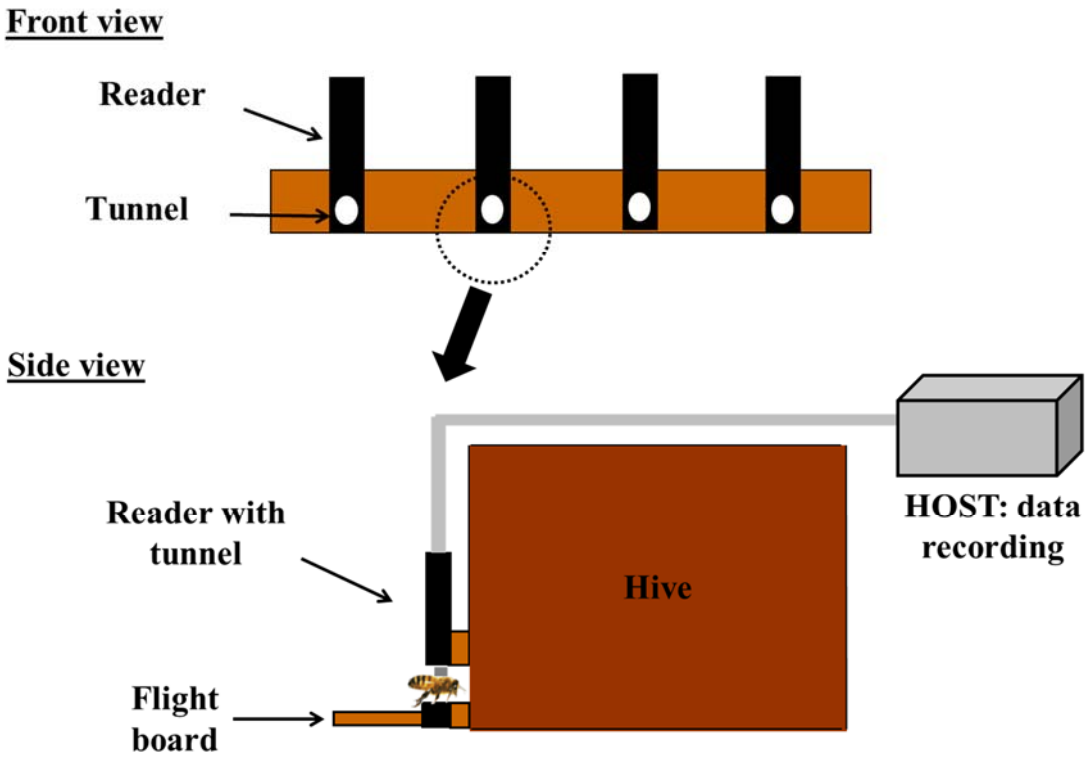


Figure 1: Picture of the RFID device

The tag's identification code (Unique identifications, or UIDs) and the exact time of the event (date, hour, minute and second) were recorded with the MAJA Host capture software. RFID data were collected by connecting the MAJA Host with a PC laptop equipped with Microsoft Mobile Device Center software. The date and time (hour, minute and second) settings for the MAJA Host and PC laptop were synchronized before data collection. To maintain continuous recording, power supply was required, via either battery or electricity.

Reading rates: The acceptance criterion for the reading was that **at least 95% of the crossing of bees should be recorded**. To ensure that this was possible, a performance check was conducted – before the system was fitted to the hive – by simulating honeybees crossing with tags glued onto small plastic or wooden sticks.
Protocol to control the performance of the RFID system and results of the RFID-reading rates 2018 and 2019 of the ring test group are presented in **Appendix 2**.

Fitting RFID equipment to the colonies: The first experimental hive was equipped with the RFID device at least **two days before the test**. For the other test colonies, a blank platform which mimicked the RFID system was placed at the hive entrance to allow the forager bees to familiarise themselves with the entrance style prior to fitting the RFID readers for the experiment.

Tag Batches: Pre-numbered 'Tag Kits' were used to tag the bees. Each kit contained RFID Tags which had previously been read and identified using a Pen reader to identify the UIDs of the tags in a particular kit. This information was stored in an excel spreadsheet. The kits were then allocated to a particular treatment group. This allowed the UIDs and hence the bees and kits to be tracked. Three to four batches of 10 to 15 tags (bees) were prepared per each test run and treatment.

4.3 Test item

Technical grade neonicotinoid active ingredient (a.i.) thiamethoxam
Supplier: Laboratories Dr. Ehrenstorfer-Schäfers, Augsburg
CAS number: 153719-23-4
Purity> 99.0%

All participating labs used technical grade thiamethoxam with the same batch number. Certificate of analysis 2018 and 2019 are proposed in **Appendix 3**.

4.4 Test design

Number of treatments:	1 control group and 3 test item groups
Number of bees labelled and exposed per treatment and run:	minimum of 30 bees ➔ 3 cages of 10 tested bees respectively for 30 bees tested (the cage is the experimental unit)
Number of test runs:	3, each one with a different colony

4.5 Preparation of the test item and test feeding solutions

The preparation of the test item and test feeding solutions is presented in **Appendix 4**.
Test item solutions: Stock solutions of the test item were prepared. These could be prepared in advance and stored in the refrigerator at 4 °C ± 4 °C for up to 5 days before the start of the test. Acetone was used as solvent. An untreated solvent control using acetone was prepared (purity ≥ 98%). The final volume of the test or control solutions in the sucrose feeding solution was 0.1% (v/v). It was previously shown, that up to a concentration of 1% acetone in sucrose solution had no significant effect on homing success of bees compared to bees receiving only untreated water (see final reports of the ring tests 2015 and 2016/2017).

Test feeding solutions: The test item and control solutions (acetone 0.1 % v/v) were administered in a sucrose feeding solution containing 30% (w/v) sucrose in demineralised water, corresponding to 30 g sucrose in 100 ml of demineralised water.

Test feeding solutions could be prepared up to 1 day before the test and stored at 4 °C ± 4 °C.

Test item, control and sucrose solutions were prepared fresh for each test run and stored in a deep freeze at –20°C ± 2°C after treatment of the bees for analytical determination of the actual treatment concentrations and dose per bee. To do so, the 3 treated test feeding solutions (≥ 5 ml of solution per sample) of each 3 test runs were forwarded to ITSAP laboratory before being sent to the French food safety agency (ANSES, Sophia Antipolis) for subsequent analyses.

4.6 Test cages

All laboratories used ventilated cages of an appropriate size for the number of captured foragers. For a good observation and handling of the tested bees, cages were designed appropriately, (either having transparent panels or being completely transparent) and could be opened easily to allow insertion and release of the bees.

4.7 Homing flight test procedure

Capture and preparation of "powdered" foragers
In the morning of the test day when the bees were actively foraging, returning foragers either with or without pollen were captured at the hive entrance.

In 2019, as far as possible, only pollen foragers were preferentially captured at the hive entrance. The percentage of pollen foragers on the total captured bees was recorded (**Appendix 5**).

Two equivalent methods were used to collect foragers:

1) One by one with entomological clamps
Bees were collected with entomological clamps (up to 300 individuals per group) and were placed in boxes (e.g. plastic food trays of 600 to 2000 cm^3 with for example 11 x 15 x 12 cm height). Bees were introduced in each box through a hole in the lid, which could be covered to prevent escape.

2) Collectively with an insect aspirator or other suitable system
Bees were collected with an insect aspirator or other system in containers (e.g. plastic bottles of 1000 cm^3 or boxes). Containers (bottles or boxes) were weighed empty and then weighed with the bees captured using a field precision balance (e.g. max 500 g, precision 0.1 g). The weight of bees was converted into the number of bees. To do so, **a group of 20 foragers of the experimental colony** were weighed to estimate the mean weight per bee.

A minimum of 600 returning bees carrying pollen were collected at the hive entrance and kept in boxes of max. 300 bees. Hydrophobic Powder (pink fluorescent pigments – T series, COLOREY SAS, France) was added in each box containing captured bees with a proportion of **0.3 mg per bee (e.g. 30 mg for 100 bees)**. Boxes/bottles were gently shaken in order to color the bees evenly. A preliminary acute toxicity study performed in 2016 showed that the pink hydrophobic powder alone or in combination with the tested test item doses did not lead to adverse effect on survival, sensitivity and natural behaviour of foragers compared to non-powdered bees exposed or not to the tested doses of thiamethoxam (see the report "Summary of the results of the international ring test 2016 and 2017").

Before or after being marked with the colored powder, collected foragers were transported to a release site located at 1 km (+/- 100 m) away from the experimental colonies. The release point was selected at a certain distance (e.g. 20 meters) from a landscape barrier (e.g. not in front of a hedge) for the bees to correctly fly away and return to the colony. Boxes/bottles were placed on a flat surface and opened to allow the bees to exit. If necessary, the bees were emptied out.

Recapture of the "powdered" foragers at the hive entrance
Recapturing of foragers: Colored bees returning to the hive were collected (on the flight board) up to a maximum of 2 hours following release (**Appendix 1**). Thus, bees captured had at least one homing experience to the hive from the release site. Bees were grouped into cages (up to 50 bees per cage) with food *ad libitum*. **Candy (**e.g. Apifonda®) **was used**. If necessary, water could also be provided once the bees were captured.

Number of foragers captured: A minimum of 140 foragers must be captured to obtain at least 30 foragers per treatment group.

Labelling and exposure in the laboratory
Feeding, starvation and labelling phase:
Captured foragers were transferred to the laboratory (holding a constant temperature of 23 ± 3°C during the entire experimental phase). Foragers were first provided with food *ad libitum* (candy: e.g. Apifonda®) for one hour to synchronize their dietary state. During this feeding period, cages were kept in dark conditions (e.g. half opened isolated box with a wet towel to avoid dehydration).Water could also be provided for the bees during this period.

After this period, the test started and the bees underwent a starvation phase of **90 mins duration (1.5 hours)**. During the starvation phase, the bees were transferred one by one from the cages to a holding cage (e.g. queen marking device, **Appendix 1**) where the bees were fixed and immobilised by a foam plunger without damage. Then, a RFID tag was glued with dental cement (e.g. Temposil®, Coltene) on the dorsally side of the thorax of each bee. Dental cement is non-corrosive and dries very quickly (less than 2 minutes).

During the labelling phase, the glue dispenser was placed in crushed ice when not in use to avoid the dental cement drying and blocking the tip. The labelling was performed without using anaesthetic on the bees.

The RFID tags were registered and allocated per treatment beforehand (cf. 4.3 RFID device). After labelling, the foragers were transferred in groups of 10 to 15 bees into cages (minimum of 3 cages of 10 bees per treatment). The cages with the RFID labelled bees were kept in the dark until the exposure phase.

Exposure phase: The test was conducted with three test item doses and one untreated control treatment (see table below). From 2018, tested sublethal doses were changed to take into account possible differences in sensitivity of the bees around the dose of 1-ng per bee. The two highest doses would correspond to a NOEL ('No Observed Effect Level') on mortality, 48-h after exposure.

Treatment	Test item doses
1	Untreated control (acetone 0.1 % (v/v)
2	0.33 ng per bee (acetone 0.1 % (v/v)
3	1 ng per bee(acetone 0.1 % (v/v)
4	1.5 ng per bee (acetone 0.1 % (v/v)

Exposure procedure: The honeybees were exposed by feeding them with 20 µl per honeybee (200 µl per group of 10 bees) of the 30% (w/v) sucrose solution containing the test item at different concentrations and the control solution (treatments). The volume of the treatments was provided using a feeder system enabling contact with the food only through the mouth parts (e.g. the tip of a micropipette bevelled). The bees in each cage shared the feeding solution via trophallaxis.

Exposure conditions: The minimum exposure duration was 1 hour in dark conditions for all treatments to limit the stress. If bees in some cages did not consume all the provided treatment solution within one hour, the exposure phase was prolonged for all bees and treatment groups for a maximum of 30 min or until all bees had consumed the sucrose solution within this time (max. exposure phase 1.5 hours). The start and end time of exposure duration was recorded.
Remaining treatment solution in any of the test cage feeders after the max. exposure phase of 1.5 hours, was measured by weighing the feeders in order to calculate the actual volume and dose consumed per bee. To do so, volume of sucrose solution prepared was weighed each time before the exposure phase for density determination.

Post-exposure: After exposure phase (60 min. (minimum) to 90 min. (maximum)), honeybees underwent an additional 40 minutes starvation period in the dark. During post-exposure starvation, cages were kept in an isolation box (e.g. cooler) including a wet towel in order to avoid dry up (the lid should be half-open).

Mortality and lost tags: During the release phase, any dead bee and lost tags were collected, identified (thanks to the UID of the tag) and recorded. They were excluded from any homing performance calculations. As a validity criterion, mean mortality of control bees over all replicates should not to exceed 15%

Honeybees release
Transport: The treated honeybees were transported **to the same release site as** at the first time after coloring at 1 km (+/- 100 m) from the colony
Temperature and humidity levels during transport were maintained to ensure their safe keeping, particularly if the release place is far away from the laboratory (transport of the bee cages in cool boxes containing a damp cloth, in a box incubator…)

Before release: the cages representing the tested treatments were put on a flat surface at least a few centimeters off the ground, and then opened simultaneously. If necessary, the bees were emptied out.

At release time, weather conditions should be favourable for foraging activity (wind below 5 of Beaufort scale, temperatures of at least 15°C and no rain).
Release start and end time (hour and minutes), local weather conditions (temperature and hygrometry (%)) were recorded during the release phase. Cloud cover and wind strength were also estimated (**Appendix 6)**.

Release time and homing flight recording: release time was **at least two hours** before sunset to allow foragers enough time to fly back to the hive. The homing flight of tagged bees was recorded during 24-h after release.

4.8 Test Schedule

Bee powdering, capture, labelling, exposure and release phases for the test took place over one day.

The homing flight recording of the labelled foragers started immediately after the release and continued for 24 hours. This 24h-recording period was sufficient for assessing the homing success of released bees (Henry et al. 2012, homing flight ring test results 2015 to 2019).

4.9 Assessment

The data recorded with RFID readers for the bees returning to the hive: Following raw data were recorded in electronic form (MAJA Host storage system equipped with the appropriate software via PC connected to the host): the UID of the tag, the reader number and the reading time (date, hour, minute and second). Data were collected for 24 hours after the release.

The weather conditions: temperature (T°C) and hygrometry (%) were recorded at least once per hour using a data logger placed under the tested hive with RFID system. Rainfall (mm) per day was also recorded at the same place using a rain gauge.

Landscape: a map of the area with location of the tested colonies and release site labelled on the map (e.g. Google Earth map) was established. The GPS coordinates of the colonies location and of the release point were given or indicated on the map. From the map, landmarks (roads, hedges, buildings, rivers…) that the bees can cross when returning to the hive were counted as a measure of landscape complexity. Indeed, Henry et al. 2014 showed that exposed bees had lower homing performances with higher number of landmarks, that is, when landscape is more complex. The counting was performed on a trajectory more or less linear from the release site to the hive (deviation of about 20°on both sides of the release point) according to the results of Fisher et al. (2014).

After the ring test 2018, **a questionnaire** was proposed to each lab to try to identify and solve critical points and problems with the homing flight method.

4.10 Summary of the test procedure changes in 2018 and 2019

	2018	**2019**
Type of foragers first collected (before being colored)	All returning foragers to the hive (pollen or nectar)	Focus as far as possible on pollen collectors returning to the hive
Pre-exposure starvation	1h30	1h30
Exposure phase	1h00 (1h30 maximum)	1h00 (1h30 maximum)
Post-exposure starvation	40 mins	40 mins
Feeding *ad libitum* before release	No	No

4.11 Results presentation and statistical analysis

- **Homing success and homing duration**

After labelling and before release in the field, the number of dead bees was used to calculate a mortality rate per treatment for each test run.

Homing performance was characterised by two variables:

- The homing flight success (**main variable**), which is a binomial variable with a value of 1 if the honeybee returns to the hive and is recorded over the 24-hours period, or 0 if it does not return.
- The homing time 24 hours after release (**secondary variable**), which is a quantitative variable. For each honeybee, it is defined as the time between the release and the first recording when passing the readers (entering the hive).

Data files organisation and statistical analysis were performed using the software R version 3.3.1 (R Development Core Team, 2016). Homing success and its duration was determined from three files: **1.** honeybee identification and treatment allocation (with UID of the tags), **2.** information at the release place (date, hour and minutes of release), **3.** RFID recording at the hive entrance. The three data sets were used to provide one raw data file per identified honeybee and treatment where homing time was expressed in minutes. During the 24 hours of RFID recording, a honeybee can be recorded several times when it passes the RFID reader (in or out the hive) for foraging activities. Therefore, several data points were recorded and can be calculated for the same bee. We only used the shortest homing time per bee, which corresponds to the first recording of the tagged bees at the hive after release. A bee which didn`t return to the hive after release was missing in the raw data, and was identified due to the missing UID in the raw data when compared to the registered UIDs before release.

One raw data file was created per run and all three runs pooled for the data analysis. Data from the three test runs were pooled to maximize the total number of bees per treatment (total of ≥ 90 bees labelled with a RFID tag) for the homing test analysis including data structuration and statistical treatments (Henry et al. 2012). The results per run or for the pooled data are presented as cumulative homing probability of the bees to the hive over the 24-hours period per test item treatment and control group. Homing duration was illustrated as boxplots (medians, quartiles).

From the results of the three test runs (pooled data), the bee homing rates back to the hive obtained over the 24-hour period for each treatment were compared using a Chi^2 test ($P < 0.05$). An adjusted significance threshold was applied for paired comparisons with Bonferroni method. Concerning homing duration, data normality and homogeneity of variance were first tested with a Shapiro-Wilk test and a Bartlett test respectively ($P > 0.05$). As data didn't show normal distribution and/or variance homogeneity, homing durations obtained were compared between treatments using a non-parametric Kruskal-Wallis test ($P < 0.05$) followed by a Mann-Whitney test for paired comparisons. An adjusted significance threshold was also applied for paired comparisons with Bonferroni method.

From the test data analysis, we determined a 'No Observed Effect Dose' (NOED) on the homing flight. The NOED was expressed as **ng test item per honeybee**.

- **Analysis of homing results variability**

In order to assess the effects of different factors on the homing performance ($P< 0.05$), we used generalized linear mixed-effects models (GLMMs) with a logit link function using the R package lme4 (Bates et al., 2018). We considered test runs' data of all the labs. The homing flight was considered as a binary response variable (0 = no return, 1 = return during the 24 hours of recording). The identity of experimental colonies and of the release sites were included as random variables. The real exposure dose was introduced as a fixed, quantitative, explanatory variable. Additional explanatory variables were temperature (punctual temperature at the release time), Varroa mite infestation of the colonies (number of varroa per 100 bees) and landscape (number of landmarks that bees can cross during the returning to the colony as an indicator of landscape complexity).
All the possible two-way interactions among explanatory variables were considered within the frame of a multimodel inference procedure (Burnham and Anderson, 2002) using the R package MuMIn

(Barton, 2018). The multimodel inference produces a single global model by averaging coefficients of explanatory variables within a set of simpler models with respect to each model's relative weight of evidence. The weight of evidence of a simpler model based on the Akaikee information criterion (AIC), gives the probability that the model is the best one in the model set, considering a parsimony tradeoff between fit and complexity. We restricted the multi-model inference to the sub-set of best models with 95% chance of including the most parsimonious combination of explanatory variables.

Each explanatory variable was standardized beforehand to the range [0,1] by subtracting each datum point from the minimum value divided by the maximum value minus the minimum value. Then, variable values were readily interpretable in terms of size of effect and were comparable among each other. Data for Varroa and landscape variable were log10-transformed.

4.12 Validity criteria of the study

Validity criteria were considered as:

- Mortality rate in control bees after exposure and before release ≤ 15 % for each test run
- The minimum and acceptable homing success rate of control bees for each test run of at least 60% over the 24 hours period.

5-RESULTS AND DISCUSSION

Eight laboratories out of 10 could perform the test in 2018 and 2019. 24 and 23 test runs were conducted in 2018 and 2019, respectively. Administered volumes of control and treated sucrose solution to the caged bees during exposure phase were totally consumed each time, for each treatment and run.

5.1 Mortality before release

Bee mortality was recorded from the end of the exposure phase until the release phase in the field.

Tables 1 to 6 present numbers of foragers labelled and released in 2018 and 2019. Bees not released include dead bees and/or bees that lost their tags. Mortality before release was generally low and met the validity criterion (**dead bees ≤ 15 %**) for control bees (except for one run in 2019) but also for 0.33 and 1-ng exposed bees.
Some lethal effects (**dead bees > 15 %**) could punctually appear especially for the bees exposed to the highest dose of 1.5 ng per bee. For 1.5 ng exposed bees, run 1 of Lab1, run 2 of Lab 5, run 3 of Lab 2 are concerned in 2018; run 1 of Lab 8, run 2 of Lab 7, run 3 of Lab 6 and Lab 7 in **2019** (Figures 2 to 7).

Table 1: Number of labelled (LB) and released bees (RB) for the test run 1 in 2018

Lab	Bee race	Nb of bees	Control	0.33 ng/bee	1 ng/bee	1.5 ng/bee
1	Carnica	LB	30	30	30	30
		RB	28	28	27	23
2	Ligustica	LB	30	30	30	30
		RB	30	30	30	30
3	Carnica	LB	30	30	30	30
		RB	30	30	20	30
4	Buckfast	LB	30	30	30	30
		RB	29	28	26	24
5	Ligustica	LB	30	30	30	30
		RB	25	27	28	27
6	Black x Buckfast	LB	40	40	40	40
		RB	40	39	39	39
7	Buckfast	LB	30	30	30	30
		RB	29	29	30	28
8	Carnica	LB	42	42	42	42
		RB	41	38	42	37

In red, results for which dead bees > 15 %

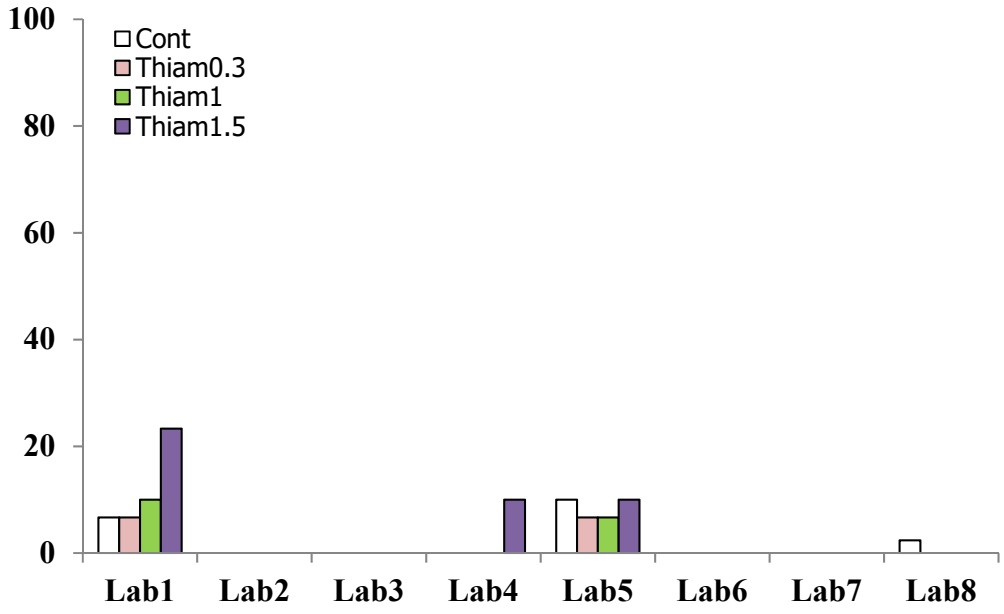


Figure 2: Mortality rate (%) before release for the test run 1 in 2018

Table 2: Number of labelled (LB) and released bees (RB) for the test run 2 in 2018

Lab	Bee race	Nb of bees	Control	0.33 ng/bee	1 ng/bee	1.5 ng/bee
1	Carnica	LB	30	30	30	30
		RB	28	28	30	30
2	Ligustica	LB	30	30	30	30
		RB	29	29	30	30
3	Carnica	LB	40	40	40	40
		RB	39	38	38	40
4	Buckfast	LB	30	30	30	30
		RB	30	30	28	30
5	Ligustica	LB	30	30	30	30
		RB	22	17	23	20
6	Black x Buckfast	LB	40	40	40	40
		RB	39	39	40	34
7	Buckfast	LB	30	30	30	30
		RB	30	30	30	29
8	Carnica	LB	42	42	42	42
		RB	41	40	42	40

In red, results for which dead bees > 15 %

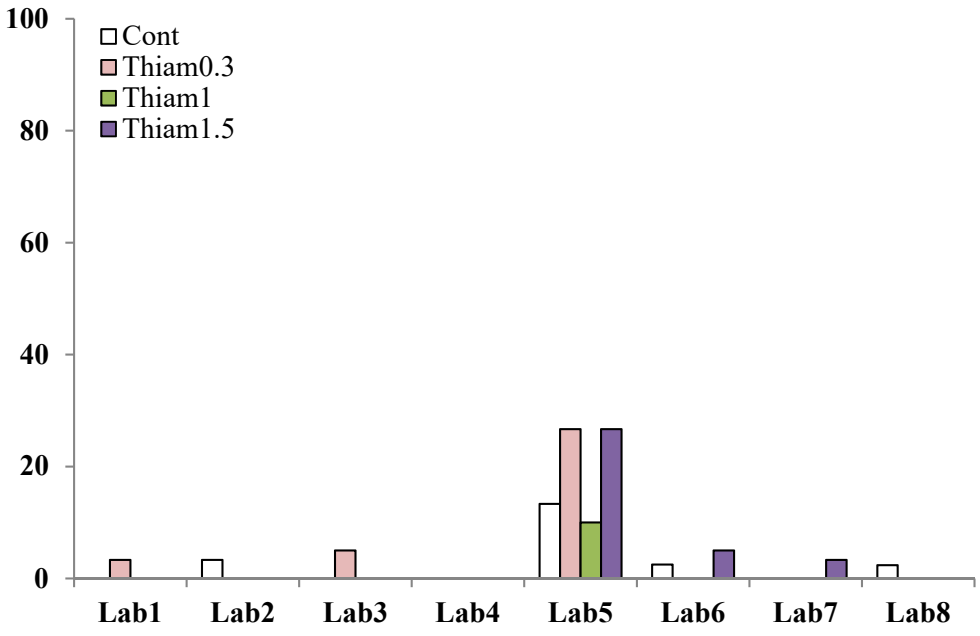


Figure 3: Mortality rate (%) before release for the test run 2 in 2018

Table 3: Number of labelled (LB) and released bees (RB) for the test run 3 in 2018

Lab	Bee race	Nb of bees	Control	0.33 ng/bee	1 ng/bee	1.5 ng/bee
1	Carnica	LB	30	30	30	30
		RB	30	30	30	28
2	Ligustica	LB	30	30	30	30
		RB	30	30	30	12
3	Carnica	LB	40	40	40	40
		RB	37	37	40	37
4	Buckfast	LB	30	30	30	30
		RB	30	29	24	22
5	Ligustica	LB	30	30	30	30
		RB	25	29	23	28
6	Black x Buckfast	LB	40	40	40	40
		RB	37	30	35	33
7	Buckfast	LB	30	30	30	30
		RB	30	30	30	28
8	Carnica	LB	42	42	42	42
		RB	40	39	41	41

In red, results for which dead bees > 15 %

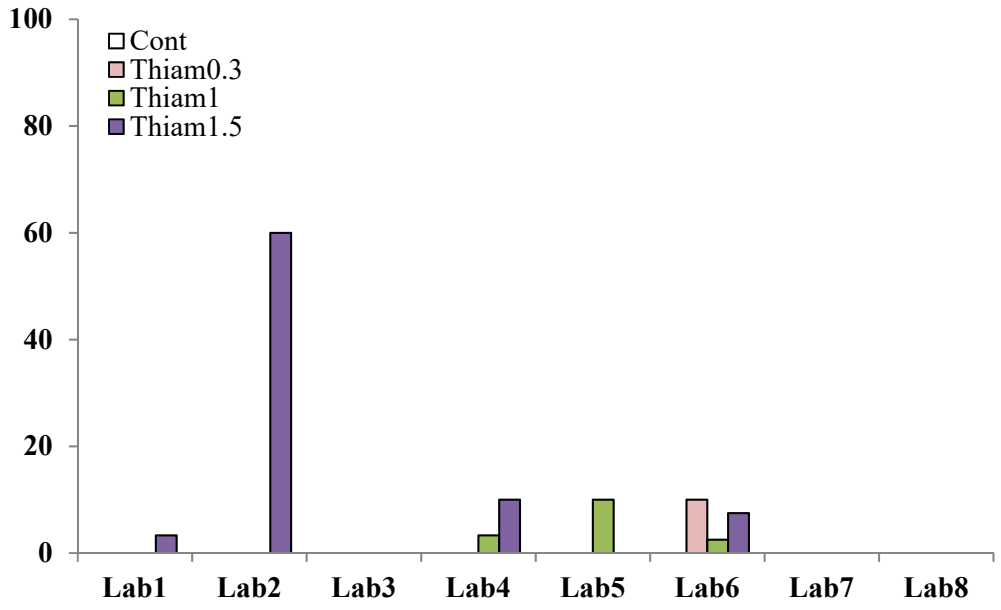


Figure 4: Mortality rate (%) before release for the test run 3 in 2018

Table 4: Number of labelled (LB) and released bees (RB) for the test run 1 in 2019

Lab	Bee race	Nb of bees	Control	0.33 ng/bee	1 ng/bee	1.5 ng/bee
1	Buckfast	LB	30	30	30	30
		RB	27	28	25	27
2	Ligustica	LB	30	30	30	30
		RB	30	30	30	25
3	Carnica	LB	30	30	30	30
		RB	28	30	30	28
4	Carnica	LB	40	40	40	40
		RB	39	37	38	39
5	Black x Buckfast	LB	40	40	40	40
		RB	39	39	39	39
6	Buckfast	LB	30	30	30	30
		RB	29	30	30	29
7	Ligustica	LB	30	30	30	30
		RB	25	25	23	24
8	Carnica x Buckfast	LB	40	40	40	40
		RB	32	36	32	33

In red, results for which dead bees > 15 %

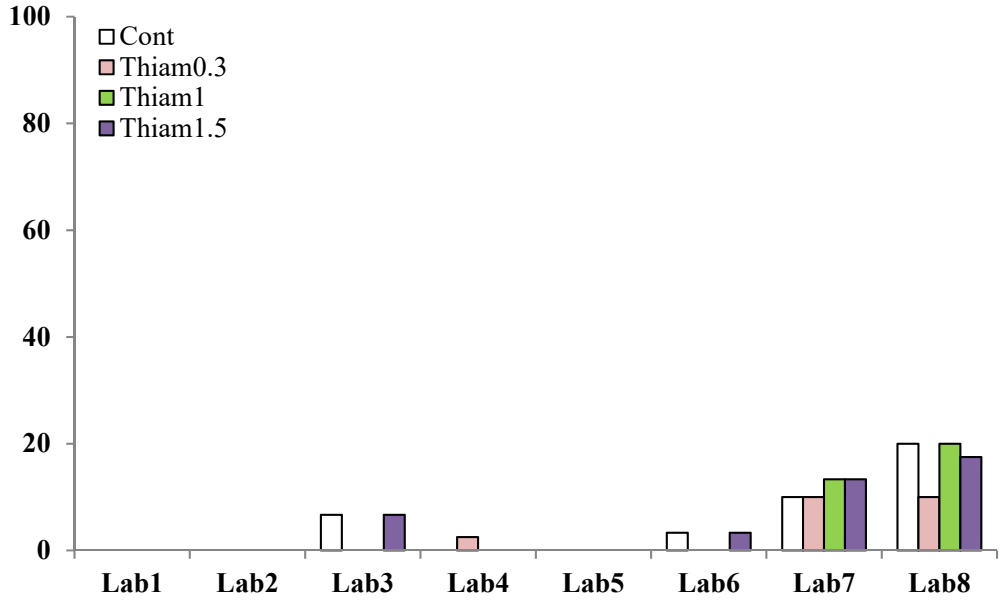


Figure 5: Mortality rate (%) before release for the test run 1 in 2019

Table 5: Number of labelled (LB) and released bees (RB) for the test run 2 in 2019

Lab	Bee race	Nb of bees	Control	0.33 ng/bee	1 ng/bee	1.5 ng/bee
1	Buckfast	LB	30	30	30	30
		RB	29	28	29	25
2	Ligustica	LB	30	30	30	30
		RB	29	30	30	26
3	Carnica	LB	30	30	30	30
		RB	27	25	24	27
4	Carnica	LB	40	40	40	40
		RB	40	39	37	40
5	Black x Buckfast	LB	40	40	40	40
		RB	38	37	38	37
6	Buckfast	LB	30	30	30	30
		RB	30	30	30	28
7	Ligustica	LB	30	30	30	30
		RB	24	24	23	21
8	Carnica x Buckfast	LB	40	40	40	40
		RB	35	34	32	30

In red, results for which dead bees > 15 %

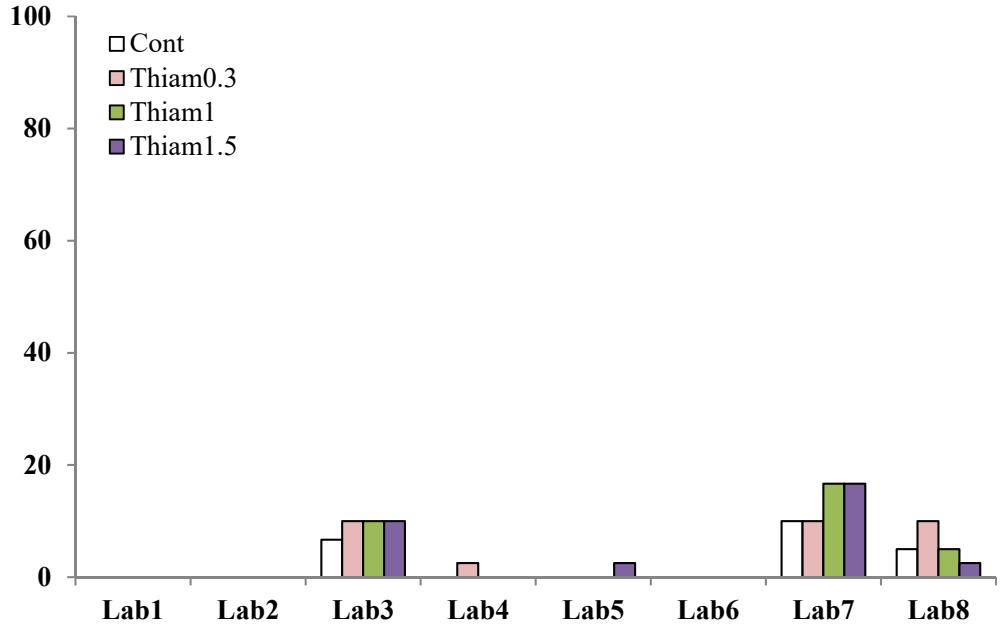


Figure 6: Mortality rate (%) before release for the test run 2 in 2019

Table 6: Number of labelled (LB) and released bees (RB) for the test run 3 in 2019

Lab	Bee race	Nb of bees	Control	0.33 ng/bee	1 ng/bee	1.5 ng/bee
1	Buckfast	LB	30	30	30	30
		RB	28	29	27	27
2	Ligustica	LB	30	30	30	30
		RB	30	28	30	29
3	Carnica	LB	30	30	30	30
		RB	30	27	27	27
5	Black x Buckfast	LB	40	40	40	40
		RB	37	38	38	40
6	Buckfast	LB	30	30	30	30
		RB	29	29	30	24
7	Ligustica	LB	30	30	30	30
		RB	28	29	21	13
8	Carnica x Buckfast	LB	40	40	40	40
		RB	34	30	31	33

In red, results for which dead bees > 15 %

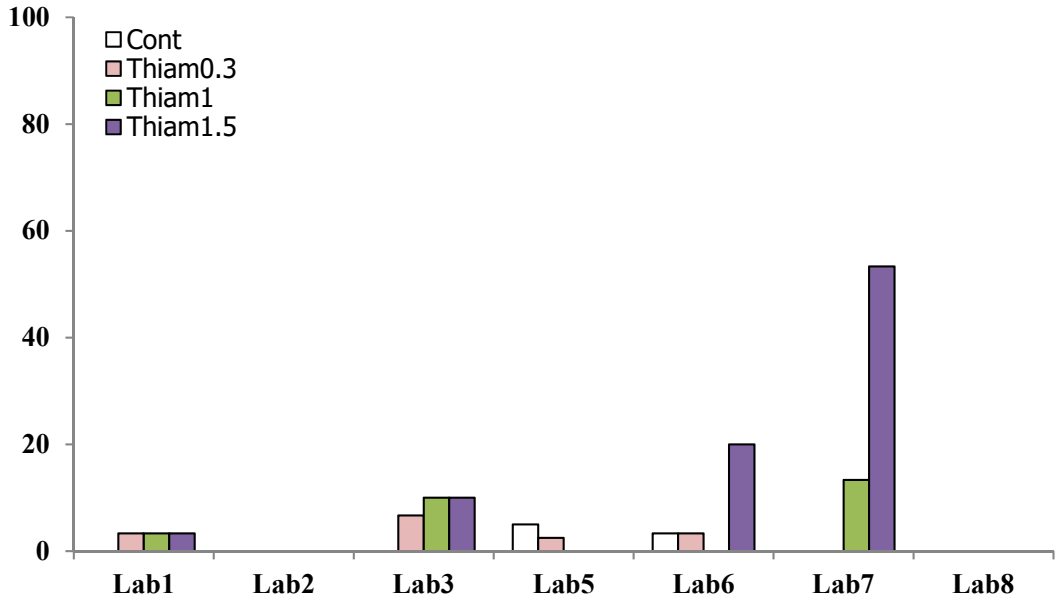


Figure 7: Mortality rate (%) before release for the test run 3 in 2019

Summary of the mortality for the five years of ring test

Over the five years of ring test, control bees' mortality ranked from 0 to 15 % in 96.8 % of the test runs (n = 125 values). Except for 4 test runs, **the validity criterion of ≤ 15% of dead bees** was met since 2015 (Table 7).

Table 7: Mortality in control bees ranked according to mortality rate classes (%) for 125 test runs performed over the five years of ring test (n= 125 mortality values for control groups)*

	[0-5[	[5-10[	[10-15[	[15-20[	[20-25[	[25-30[	[30-35[
2015	**18**	**3**	**1**	0	0	0	0
2016	**21**	**6**	**2**	0	1	0	0
2017	**21**	**2**	**1**	1	0	0	0
2018	**21**	**0**	**3**	0	0	0	0
2019	**16**	**4**	**2**	0	1	0	0

** One mortality rate ≥ 35% in 2016*

Considering treated groups, mortality decreased to ≤ 15 % in the majority of cases before release for the last three ring test years (Table 8). This could be explained by the gained experience with the manipulation of the bees in the laboratory. Then, **mortality is for instance ≤ 15 % in 94.4 %** of the cases **in 2018** (n= 72 values for all treated groups) and in **91.3 %** of the cases **in 2019** (n= 69 values for all treated groups). For these two years, only 10 values were above 15 % of mortality, and 7 of them were for the 1.5-ng exposed bees. A validity criterion of **≤ 15% dead bees** could be considered not only for control bees but for exposed bees too.

Table 8: Mortality in exposed bees ranked according to mortality rates (%) classes for 125 test runs performed over the five years of ring test (n= 375 mortality values for all treated groups)*.

	[0-5[	[5-10[	[10-15[	[15-20[	[20-25[	[25-30[	[30-35[
2015	**47**	**10**	**3**	**4**	1	1	0
2016	**68**	**9**	**6**	3	2	2	2
2017	**63**	**7**	**3**	0	1	0	0
2018	**55**	**6**	**7**	0	0	2	1
2019	**46**	**3**	**14**	3	2	0	0

**Four mortality rates ≥ 35%: one in 2016 for the 0.1-ng exposed bees; one in 2017 for the 1-ng exposed bees; one in 2018 and one in 2019 for the 1.5-ng exposed bees*

5.2 Homing performance in control bees

Summary of the homing performances in control bees for the five years of ring test

For a majority of test runs, homing performance in control bees ranked from 60 to 100 %. It also progressed from 70 to 100 % in 2017 and 2019 (Table 9).

According to the ring test results the **minimum and acceptable homing performances in control bees vary from 60 to 70 %, which might be used as a validity criterion**.

Table 9: Homing performances in control bees ranked according to homing rate (%) classes for 125 test runs performed over the five years of ring test.

	2015	2016	2017	2018	2019
Homing rate classes for control bees (%)	**Nb of tests**	**Nb of tests**	**Nb of tests**	**Nb of tests**	**Nb of tests**
[0-10[	0	0	0	2	1
[10-20[	0	1	0	0	0
[20-30[	0	1	0	1	1
[30-40[	2	0	0	0	2
[40-50[	0	0	0	2	2
[50-60[	3	4	3	2	1
[60-70[	7	5	**3**	5	**2**
[70-80[	1	**5**	4	**4**	6
[80-90[	3	**5**	**11**	**3**	**5**
[90-100[	6	**10**	**4**	**5**	**3**
TOTAL	**22**	**31**	**25**	**24**	**23**

The percentage of valid test runs was considered with the minimum homing performance in control bees ≥ of 60, 70 or 80 % (Figure 8). Based on the results of the five ring test years, a minimum homing performance of 80% cannot be accepted because too many test runs would be invalid. Best results were obtained in 2017. However, in 2016 and 2017, bees were fed *ad libitum* before release. Feeding increased the stomach content and the effects of the insecticide (highest dose tested) could not be detected anymore due to possible dilution of the remaining stomach content origin of the exposure phase (see "summary of the results of the international ring test 2016 and 2017"). **One compromise are the results obtained in 2019**. Indeed, only 69.6 % test runs met the category ≥ 60 % (two labs performed 3 invalid test runs below 60 % of homing performances for the control bees). But when considering the category ≥ 70 %, percentage of valid test runs are relatively similar to the category ≥ 60 % with 65.2 % test runs. Only one test run was between 60 and 70 % considering the homing performance in control bees, whereas all others were ≥ 70%.

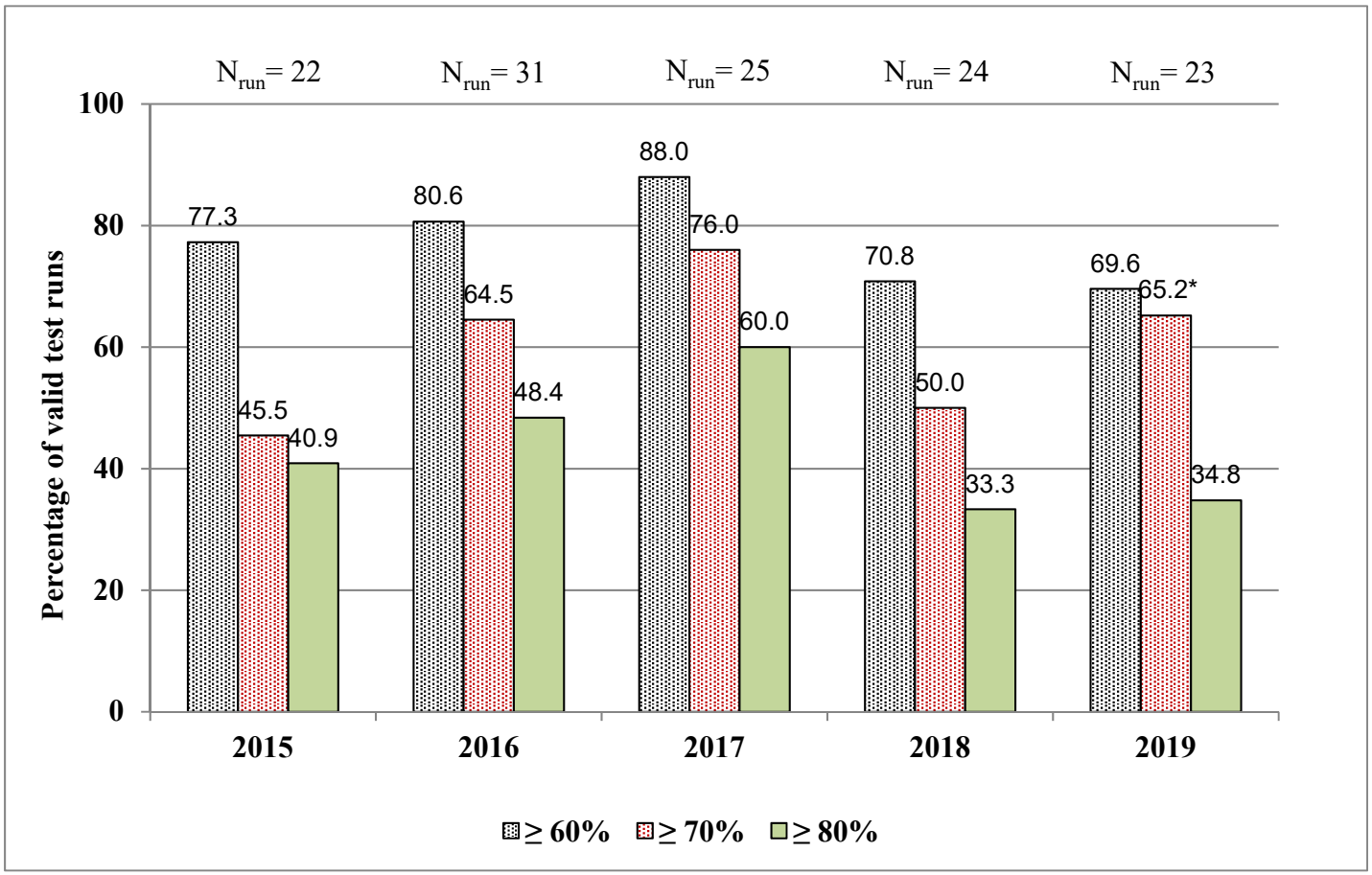


Figure 8: Percentage of valid test runs when the minimum accepted homing performances in control bees is ≥ 60, 70 or 80 %. * *One homing rate at 69 % considered in the category ≥ 70 %.*

Comparison with "natural" loss of foragers in field conditions (F. Requier, com. pers.)

Minimum and acceptable homing performances of control bees were compared to the natural" loss rates of "free-ranging foragers under field conditions. To do so, we considered a R&D study previously performed with the RFID system from April to September 2015 in the North-West of France. Groups of emerging bees from three colonies were tagged each month during the period (total: 2100 emerging bees tagged). Data were obtained for 80 % of the labelled bees. With continuous recording, bees' activity (e.g. entry and exit from the hive) could be followed each day from adult emergence to the death (no more RFID recording). Median foraging age (or median age of first foraging) was determined and calculated for each group of bees. Then, daily survival rate (%) was determined for each group of foragers and dates, from the age of first foraging to the end (when less than 10 tagged remaining bees were recorded). This survival rate (returned bees back to the colony) was calculated as number of bees recorded in the evening on day (D) / number of bees recorded in the evening the day before (D-1). All the calculated daily survival rates (%) of foragers were distributed according to Figure 9.

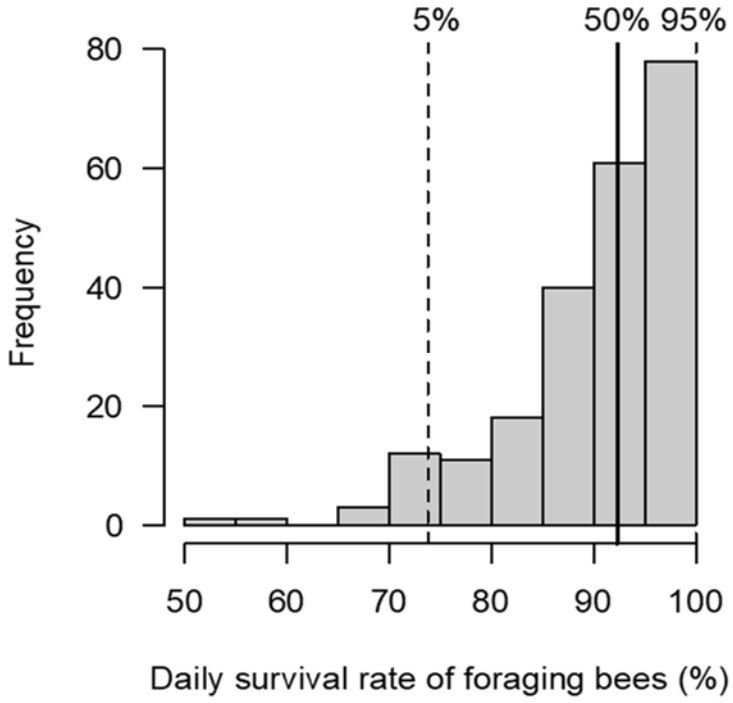


Figure 9: Ranking of all daily survival rate (%) of free-ranging foragers under field conditions. All recorded daily survival rates of foragers ranked between 50 and 100 %.

Figure 9 shows that the median daily survival rate is > 90 % but the distribution begins at 70 % (5% of the results). Then, this study would support a maximum daily foragers' loss of 30 %. This is comparable to the minimum and acceptable level of homing failure of 30 to 40 % considered for the control foragers (minimum and acceptable homing performances of 60 to 70 %).

5.2 Homing success per treatment and run

Homing performances per treatment and run were calculated 24 hours after release.

For a majority of test runs, results showed lower homing performances for the bees exposed to the highest doses (1 and 1.5-ng exposed bees) compared to control bees or 0.3 ng-exposed bees for both years (Tables 10 and 11; Figure 10).

When comparing control and 1-ng exposed bees, 4 valid runs[1] out of 17 (23.5 %) in 2018 and 2 valid runs on 16 (12.5 %) in 2019 showed low (≤ 10 %) or no differences in homing performances. **When comparing control and 1.5-ng exposed bees**, 2 valid runs out of 17 (11.8 %) in 2018 and 2 runs on 16 (12.5 %) in 2019 showed low (≤ 10 %) or no differences in homing performances (Table 10 and 11).

[1] Test run is considered valid when homing performances of control bees ≥ 60 %

Table 10: Results of homing probabilities per test run and laboratory in 2018

Lab	Run	Control	Thiam. 0.3ng/bee	Thiam. 1 ng/bee	Thiam. 1.5 ng/bee
1	1	0.750	0.893	0.556	0.000
	2	0.786	0.607	0.633	0.167
	3	0.900	0.867	0.600	0.321
2	1	0.933	0.700	0.433	0.333
	2	0.828	0.931	0.733	0.567
	3	0.633	0.600	0.367	0.333
3	*1	0.533	0.367	0.450	0.000
	*2	0.461	0.342	0.474	0.050
	*3	0.216	0.351	0.275	0.243
4	1	**0.621**	**0.821**	**0.769**	**0.542**
	2	0.833	0.700	0.607	0.500
	3	0.667	0.414	0.125	0.091
5	*1	0.040	0.259	0.179	0.074
	2	0.682	0.529	0.217	0.000
	*3	0.560	0.724	0.522	0.143
6	1	0.650	0.667	0.487	0.256
	2	0.744	0.744	0.075	0.176
	3	0.784	0.767	0.514	0.333
7	*1	0.414	0.655	0.367	0.321
	*2	0.067	0.100	0.600	0.241
	3	0.867	0.567	0.433	0.643
8	1	**0.902**	**0.895**	**0.952**	**0.622**
	2	**0.927**	**0.950**	**0.857**	**0.775**
	3	**0.900**	**1.000**	**0.976**	**0.829**

* Invalid test runs (homing success of control bees < 60%)

Runs with no or low differences in homing success between 1ng-exposed bees and control bees (≤ 10 % differences in homing performances) or homing success of 1ng-bees higher than control bees.

Runs with no or low differences in homing success between 1.5 ng-exposed bees and control bees (≤ 10 % differences in homing performances) or homing success of 1.5 ng-bees higher than control bees.

Table 11: Results of homing probabilities per test run and laboratory in 2019

Lab	Run	Control	Thiam. 0.3ng/bee	Thiam. 1 ng/bee	Thiam. 1.5 ng/bee
1	**1**	0.741	0.786	0.560	0.333
	2	0.793	0.893	0.750	0.080
	3	0.893	0.828	0.778	0.407
2	**1**	0.600	0.533	0.300	0.040
	2	0.724	0.633	0.433	0.154
	3	0.833	0.750	0.667	0.724
3	**1**	0.821	0.900	0.500	0.286
	2	0.704	0.720	0.542	0.185
	3	0.967	0.926	0.741	0.296
4	**1**	0.718	0.595	0.474	0.179
	2	0.825	0.923	0.730	0.800
5	**1**	1.000	1.000	0.795	0.846
	2	0.974	0.919	0.684	0.595
	3	0.838	0.789	0.711	0.500
6	***1**	0.345	0.367	0.267	0.207
	2	0.767	0.800	0.267	0.250
	3	0.690	0.621	0.567	0.125
7	***1**	0.520	0.400	0.000	0.000
	***2**	0.417	0.500	0.000	0.000
	***3**	0.357	0.207	0.000	0.000
8	***1**	0.060	0.111	0.125	0.121
	***2**	0.257	0.147	0.031	0.000
	***3**	0.471	0.100	0.065	0.030

* Invalid test runs (homing success of control bees < 60%)

Runs with no or low differences in homing success between 1ng-exposed bees and control bees (≤ 10 % differences in homing performances) or homing success of 1ng-bees higher than control bees.

Runs with no or low differences in homing success between 1.5 ng-exposed bees and control bees (≤ 10 % differences in homing performances) or homing success of 1.5 ng-bees higher than control bees.

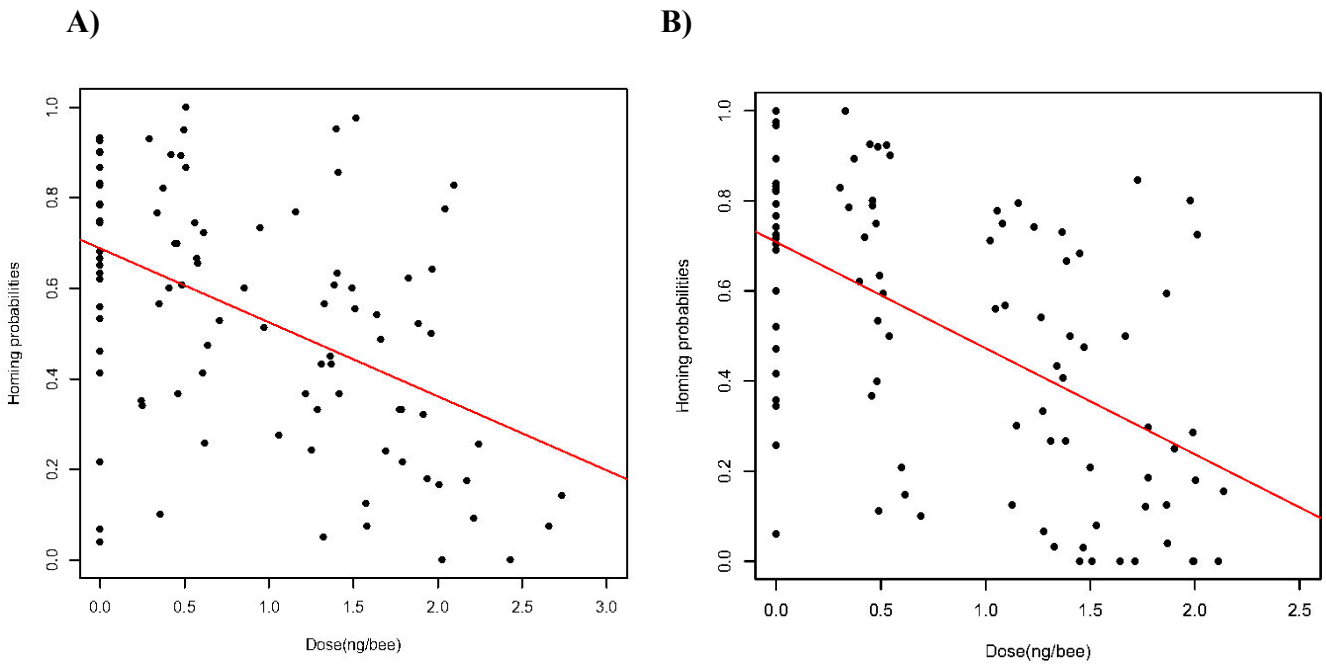


Figure 10: Relationships between homing probabilities of the foragers per test run 24 hours after release and the actual doses of exposure (ng per bee) from analyses. A red regression line was added. In 2018, two values were not included because of problems with the analytics (high and abnormal real values > 3 ng/bee, see part 5.4). **A) results obtained in 2018**, **B) results obtained in 2019**.

5.3 Climatic conditions during the tests

Climatic conditions recorded during the homing flight tests 24h-after the bees' release are presented in Tables 12 and 13 for 2018 and 2019 respectively.

In 2018, a summer heat wave occurred in July/August with difficulties to perform the test for some labs (e.g. Lab 3; Lab 4 especially for run 3). One lab couldn't perform any test at all because of problems with temperature. For Lab 3, the test was planned earlier in the season (May to July) but problems with tags delivery obliged to delay the test to August. Then for this lab, higher temperatures encountered have played a role in the lower homing performance as they faced in 2018.
For other labs, temperature was not a limiting factor (questionnaires 2018). In majority, climatic conditions alone do not explain invalid test runs (homing performances in control bees < 60 %) obtained in 2018 and 2019. When rainfall was recorded the day of release, it was in general a few hours after the bees' release and the impact was limited. Based from the ring test experience, it is now well known that the great majority of bees (familiar with their environment) will return to the hive within 2 hours after release (> 90 % of the bees recorded 24-h after release).

As a whole, the homing test performance rely on favourable climate according to geographic conditions (no temperatures too low (>15 °C) but also not too high and abnormal temperature for the region) associated with blooming flowers or crops in the surroundings for foraging activities. As far as possible, the best period for the test performance is spring/beginning of summer when the colonies develop and food resources are available (high nectar and pollen flow). If nice weather conditions are experienced in the late season (e.g. August/September) with blooming flowers/crops , test can be performed but making sure that Varroa pressure is low (see part **5.7**).

Table 12: Mean climatic conditions recorded during 24 hours after bees' release for each laboratory and run in 2018

Lab	Run	Mean temperature (°C)	Mean hygrometry (%)	Mean rainfall (mm)
1	1	22.97	57.22	0
	2	25.15	72.60	0
	3	23.9	86.19	0
2	1	25.31	62.10	0
	2	24.04	67.52	3[a]
	3	19.66	63.24	0
3	*1	27.19	52.53	0
	*2	24.25	55.01	0
	*3	19.72	59.56	0
4	1	29.74	57.54	0
	2	29.18	44.66	0
	3	31.66	52.26	0
5	*1	22.43	75.36	0.18
	2	26.76	62.44	0
	*3	26.17	55.84	0
6	1	18.95	78.04	0
	2	23.25	69.12	0
	3	17.20	62.72	0
7	*1	20.22	51.88	0
	*2	-	-	0
	3	22.19	-	0
8	1	21.98	87.08	13[b]
	2	19.80	54.84	0
	3	23.38	64.08	0

* Invalid test runs because of low homing success of control bees (< 60%)
[a] Storm and heavy rain after release at night (22.00 pm)
[b] Heavy rain one hour after release (duration 1h); some rain the following day

Table 13: Mean climatic conditions recorded during 24 hours after bees' release for each laboratory and run in 2019

Lab	Run	Mean temperature (°C)	Mean hygrometry (%)	Mean rainfall (mm)
1	**1**	20.52	47.92	0
	2	23.38	46.48	0
	3	25.88	58.16	0
2	**1**	18.34	64.46	0
	2	22.42	67.08	0
	3	24.60	44.80	0
3[a]	**1**	-	-	0
	2	-	-	0.7
	3	-	-	0
4	**1**	19.02	55.44	0.9
	2	19.68	68.46	0.4
5	**1**	21.74	64.27	0
	2	18.51	57.96	0
	3	21.27	70.77	0
6	***1**	18.78	82.98	0
	2	20.24	72.32	0
	3	22.62	57.26	0
7	***1**	29.87	57.45	0
	***2**	27.40	59.58	0
	***3**	28.52	61.36	0
8	***1**	22.12	49.31	0
	***2**	19.18	74.01	2.6[b]
	***3**	24.33	63.70	0

***** Invalid test runs because of low homing success of control bees (< 60%)

[a] No data for Lab 3 because of unforseen problems (no battery left for the data logger)

[b] Rainfall more than 3 hours after release (20.20 pm) for a 2.5-h duration

5.4 Analyses of the test item solutions

The test feeding solutions were analysed by the French Food Safety Agency (ANSES) laboratory (Sophia Antipolis) using the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) technique to detect real concentrations of thiamethoxam (limit of thiamethoxam quantification = 0.3 ng/mL).

In 2018, high or abnormal real values were determined as a whole. It was questionable, as there was no correlation between analytical results and mortality or homing performances results. Some sucrose solutions were re-analysed but same results were obtained. For the expected nominal dose of 1.5 ng per bee, two extreme outliers values of 3.828 and 5.142 ng per bee were determined after analytical analyses (Table 14). These two values were completely out of the dose range and were excluded for data treatment.

For the ring test 2019, it was asked to keep the stock solution prepared in acetone to analyse them in case of problems with the testfeeding solution or in case of abnormal values. In 2019, real doses analysed from sucrose solution were more concordant with what was expected (Table 15).

Table 14: Results of the analytical analyses of the test feeding solutions for each test run and laboratory in 2018

Lab	Run	Nominal dose : 1.5 ng/bee	Nominal dose : 1 ng/bee	Nominal dose : 0.33 ng/bee
1	1	5.142	1.508	0.478
	2	2.008	1.406	0.482
	3	3.828	1.494	0.510
2	1	1.772	1.368	0.442
	2	1.330	0.946	0.294
	3	1.788	1.220	0.406
3	*1	2.026	1.366	0.460
	*2	1.324	0.640	0.252
	*3	1.250	1.062	0.246
4	1	1.640	1.158	0.374
	2	1.962	1.390	0.456
	3	2.216	1.572	0.608
5	*1	2.658	1.938	0.622
	2	2.432	1.790	0.706
	*3	2.734	1.888	0.614
6	1	2.242	1.662	0.570
	2	2.174	1.580	0.560
	3	1.286	0.970	0.338
7	1	1.914	1.418	0.576
	2	1.694	0.854	0.358
	3	1.966	1.310	0.352
8	1	1.824	1.396	0.420
	2	2.042	1.408	0.498
	3	2.096	1.516	0.506

* Invalid test runs because of low homing success of control bees (< 60%)
In red: outliers

Table 15: Results of the analytical analyses of the test feeding solutions for each test run and laboratory in 2019

Lab	Run	Nominal dose : 1.5 ng/bee	Nominal dose : 1 ng/bee	Nominal dose : 0.33 ng/bee
1	**1**	1.274	1.048	0.348
	2	1.530	1.082	0.372
	3	1.368	1.056	0.306
2	**1**	1.870	1.146	0.484
	2	2.138	1.340	0.494
	3	2.012	1.386	0.478
3	**1**	1.988	1.404	0.544
	2	1.778	1.264	0.420
	3	1.778	1.230	0.448
4	**1**	2.004	1.470	0.512
	2	1.978	1.364	0.528
5	**1**	1.724	1.154	0.328
	2	1.866	1.450	0.484
	3	1.668	1.022	0.460
6	***1**	NA**	1.382	0.456
	2	1.904	1.312	0.458
	3	1.864	1.092	0.398
7	***1**	1.990	1.448	0.480
	***2**	1.994	1.644	0.540
	***3**	2.110	1.508	0.600
8	***1**	1.764	1.126	0.488
	***2**	1.714	1.328	0.614
	***3**	1.466	1.278	0.690

***** Invalid test runs because of low homing success of control bees (< 60%)
****** No sample for the analyses

5.5 Homing success per treatment

To assess homing success per treatment, data of the three test runs were pooled for labs where all three test runs fulfilled the validity criteria (when homing performance of control bees were ≥ 60 % in individual test runs). In 2019, two valid test runs where considered for one lab that could only perform two runs and for another one with one invalidated run out of three performed.

In 2018, Homing success didn't significantly differ between groups of control bees and groups of bees exposed to 0.33 ng per bee of thiamethoxam (Chi² tests; $P > 0.05$). But homing performances significantly differ between control bees and bees exposed to the highest doses of 1 or 1.5 ng per bee of thiamethoxam. Bees exposed to these highest doses returned to the hive at a significantly lower

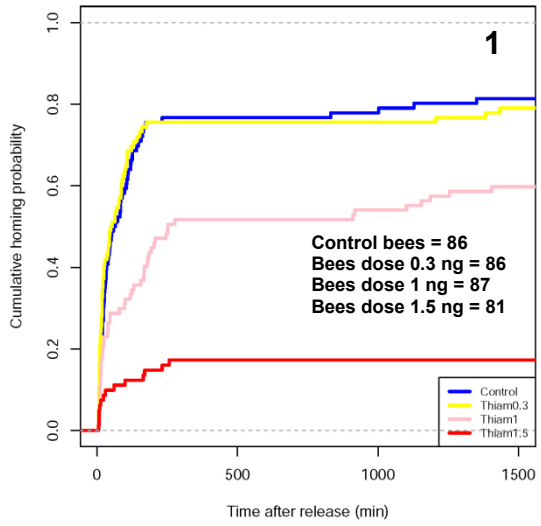
rate compared to control bees or to 0.33-ng exposed bees (Chi² tests; P < 0.05; Table 16, and Figure 11).

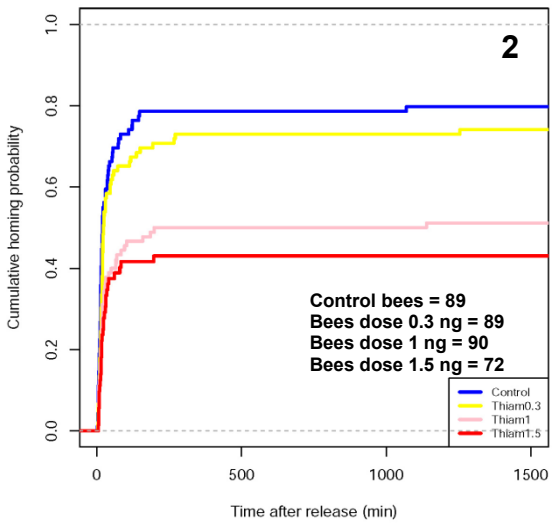
Then, a nominal No Observed Effect Dose (NOED) on the homing success of 0.33 ng per bee for 3 labs and 1 ng per bee for 2 labs could be determined.

Table 16: Homing success results for the ring test 2018 (three valid test runs pooled)

		Control	Thiam. 0.33 ng/bee	Thiam. 1 ng/bee	Thiam. 1.5 ng/bee
Lab 1	Number of foragers released	86	86	87	81
	Homing success probability (24 h after release) [1]	0.814 (a)	0.791 (ab)	0.598 (b)	0.185 (c)
	Chi² Test	Chi² = 87.716, df = 3, P < 2.2e-16			
Lab 2	Number of foragers released	89	89	90	72
	Homing success probability (24 h after release) [1]	0.798 (a)	0.742(a)	0.511(b)	0.431(b)
	Chi² Test	Chi² = 33.219, df = 3, P = 2.895e-07			
Lab 4	Number of foragers released	89	87	78	76
	Homing success probability (24 h after release) [1]	0.708 (a)	0.644 (ab)	0.513 (abc)	0.395 (c)
	Chi² Test	Chi² = 19.415, df = 3, P = 0.0002244			
Lab 6	Number of foragers released	116	108	114	106
	Homing success probability (24 h after release) [1]	0.724 (a)	0.722 (a)	0.351 (b)	0.255 (b)
	Chi² Test	Chi² = 79.931, df = 3, P < 2.2e-16			
Lab 8	Number of foragers released	122	117	125	118
	Homing success probability (24 h after release) [1]	0.910 (a)	0.949 (a)	0.928 (a)	0.746 (b)
	Chi² Test	Chi²= 29.882, df = 3, P = 1.461e-06			

[1]Pairwise comparisons were performed with Chi² test and used Bonferroni P value adjustment method. Same letters indicate no significant differences.





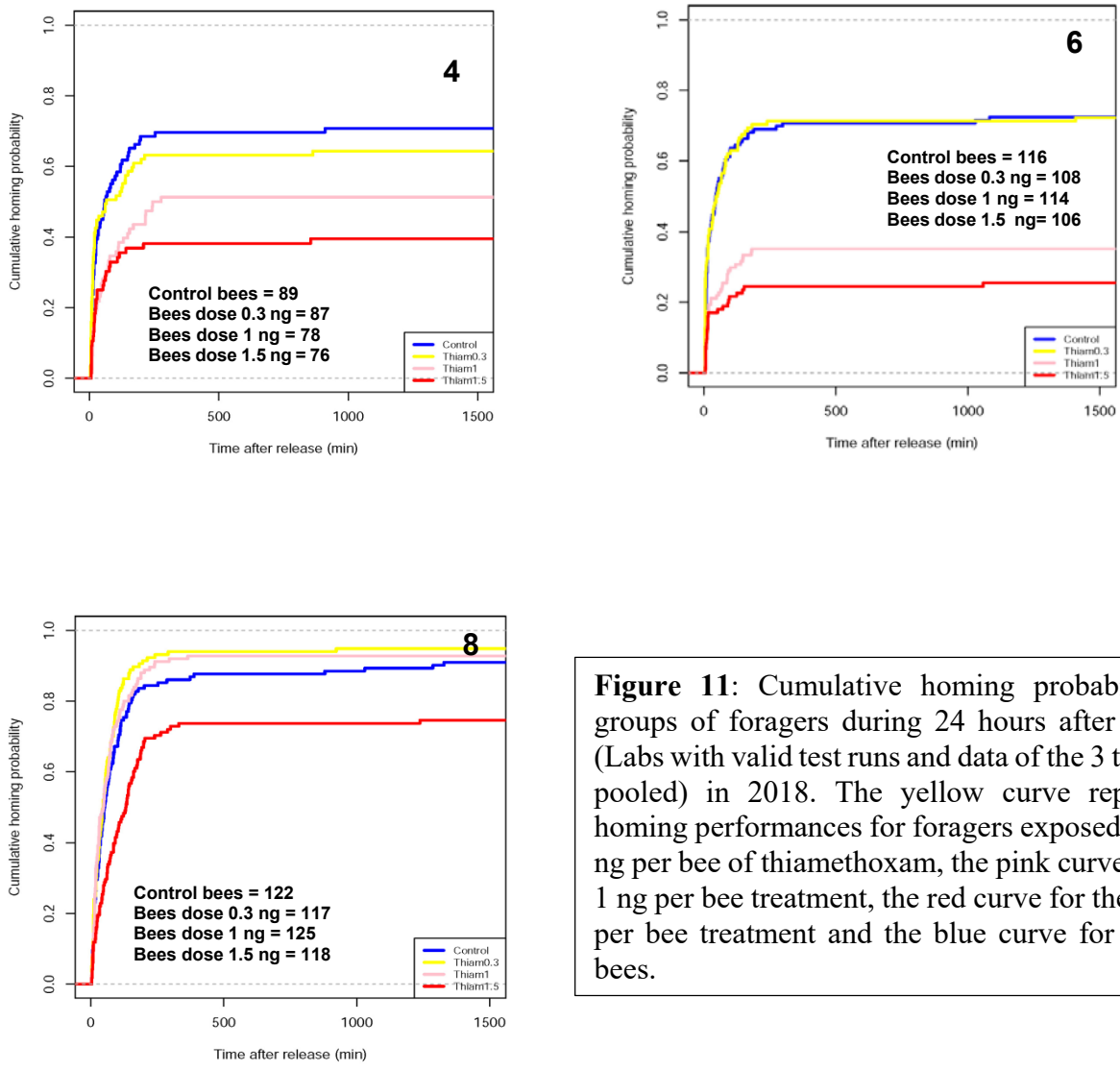


Figure 11: Cumulative homing probability of groups of foragers during 24 hours after release (Labs with valid test runs and data of the 3 test runs pooled) in 2018. The yellow curve represents homing performances for foragers exposed to 0.33 ng per bee of thiamethoxam, the pink curve for the 1 ng per bee treatment, the red curve for the 1.5 ng per bee treatment and the blue curve for control bees.

In 2019, Homing success also didn't significantly differ between groups of control bees and groups of bees exposed to 0.33 ng per bee of thiamethoxam (Chi² tests; $P > 0.05$). Homing performances significantly differ between control bees and bees exposed to the highest doses of 1 or 1.5 ng per bee of thiamethoxam. Bees exposed to these highest doses returned to the hive at a significantly lower rate compared to control bees or to 0.33-ng exposed bees (Chi² or tests; $P < 0.05$; Table 17, and Figure 12).

Then, a nominal No Observed Effect Dose (NOED) on the homing success of 0.33 ng per bee for 4 labs to 1 ng per bee for 2 labs could be determined.

Table 17: Homing success results for the ring test 2019 (two to three valid test runs pooled)

		Control	Thiam. 0.33 ng/bee	Thiam. 1 ng/bee	Thiam. 1.5 ng/bee
Lab 1	Number of foragers released	84	85	80	79
	Homing success probability (24 h after release) [1]	0.810 (a)	0.835 (a)	0.700 (a)	0.278 (b)
	Chi² Test	Chi²= 71.982, df = 3, P = 1.606e-15			
Lab 2	Number of foragers released	89	88	90	80
	Homing success probability (24 h after release) [1]	0.719 (a)	0.636 (ab)	0.467 (bc)	0.325 (c)
	Chi² Test	Chi²= 31.633, df = 3, P = 6.255e-07			
Lab 3	Number of foragers released	85	82	81	82
	Homing success probability (24 h after release) [1]	0.835 (a)	0.854 (a)	0.593 (b)	0.256 (c)
	Chi² Test	Chi² = 83.17, df = 3, P < 2.2e-16			
Lab 4*	Number of foragers released	79	76	75	79
	Homing success probability (24 h after release) [1]	0.772 (a)	0.763 (a)	0.600 (ab)	0.494 (b)
	Chi² Test	Chi² = 18.881, df = 3, P = 0.0002893			
Lab 5	Number of foragers released	114	114	115	116
	Homing success probability (24 h after release) [1]	0.939 (a)	0.904 (a)	0.730 (b)	0.647 (b)
	Chi² Test	Chi² = 42.454, df = 3, P = 3.214e-09			
Lab 6*	Number of foragers released	59	59	60	52
	Homing success probability (24 h after release) [1]	0.729 (a)	0.712 (a)	0.417 (b)	0.192 (b)
	Chi² Test	Chi² = 43.956, df = 3, P = 1.542e-09			

[1]Pairwise comparisons were performed with Chi² test and used Bonferroni P value adjustment method. Same letters indicate no significant differences. * Data from two valid test runs pooled.

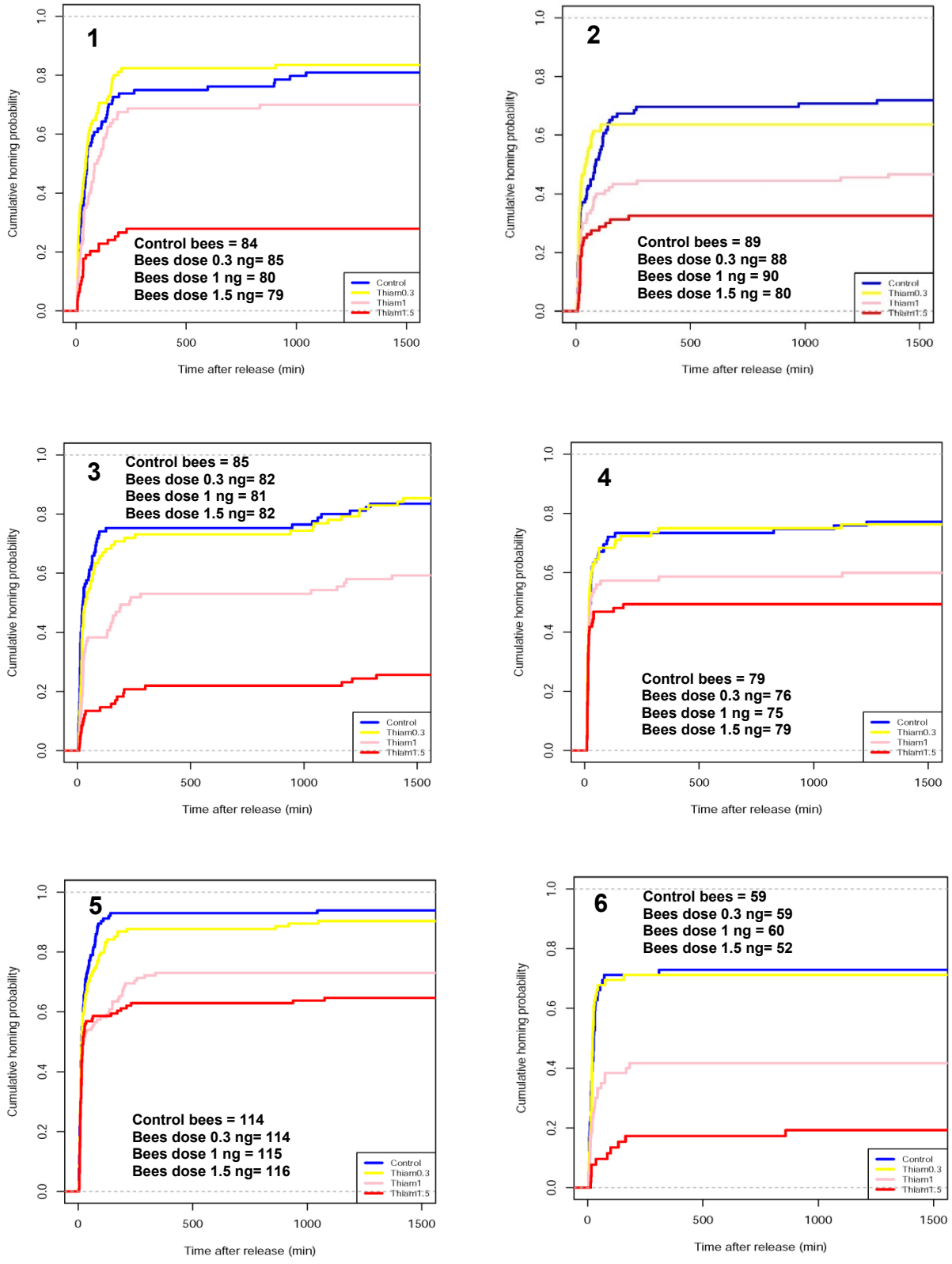


Figure 12: Cumulative homing probability of groups of foragers during 24 hours after release (Labs with valid test runs and data of 2 to 3 test runs pooled) in 2019. The yellow curve represents homing performances for foragers exposed to 0.33 ng per bee of thiamethoxam, the pink curve for the 1 ng per bee treatment, the red curve for the 1.5 ng per bee treatment and the blue curve for control bees.

Details of statistical analyses for homing performance of each laboratory are presented in **Appendix 7**.

Summary of the test endpoint (NOED) determination for the five years of ring test

As previously written, better results concerning validity were obtained in 2017. But feeding the bees before release increased the homing results variability in exposed bees and no NOED could be determined for nearly 29 % of the tests (Table 18). We confirm that the protocol applied in 2019 is the best compromise considering test validity and NOED determination.

Table 18: Percentage of tests for which a NOED could be determined or not from 2 to 3 valid test runs pooled together (homing performances of control bees ≥ 60 %)

	NOED determined (%)	No NOED determined (%)	Invalid Tests (%)
2015 (n=7 tests)	42.9	28.6*	28.6
2016 (n=11 tests)**	45.4	27.3	27.3
2017 (n=7 tests)**	57.1	28.6	14.3
2018 (n=8 tests)	62.5***	0	37.5
2019 (n=8 tests)	75.0****	0	25.0

* Labs that fed the bees after exposure and before release
** Feeding period before release in the protocol 2016 and 2017
*** NOED of 0.33 ng/bee for 60 % of the valid tests (n = 5 tests in 2018)
**** NOED of 0.33 ng/bee for 66.7 % of the valid tests (n= 6 tests in 2019)

5.6 Homing duration per treatment

As a secondary observation, we calculated homing duration per treatment 24 hours after release. Test runs data were pooled for labs where all three test runs fulfilled the validity criteria (when homing performance of control bees were ≥ 60 % in individual test runs). Like for homing performances, two valid test runs where considered for one lab that could only perform two runs and for another one with one invalidated run out of three performed in 2019.

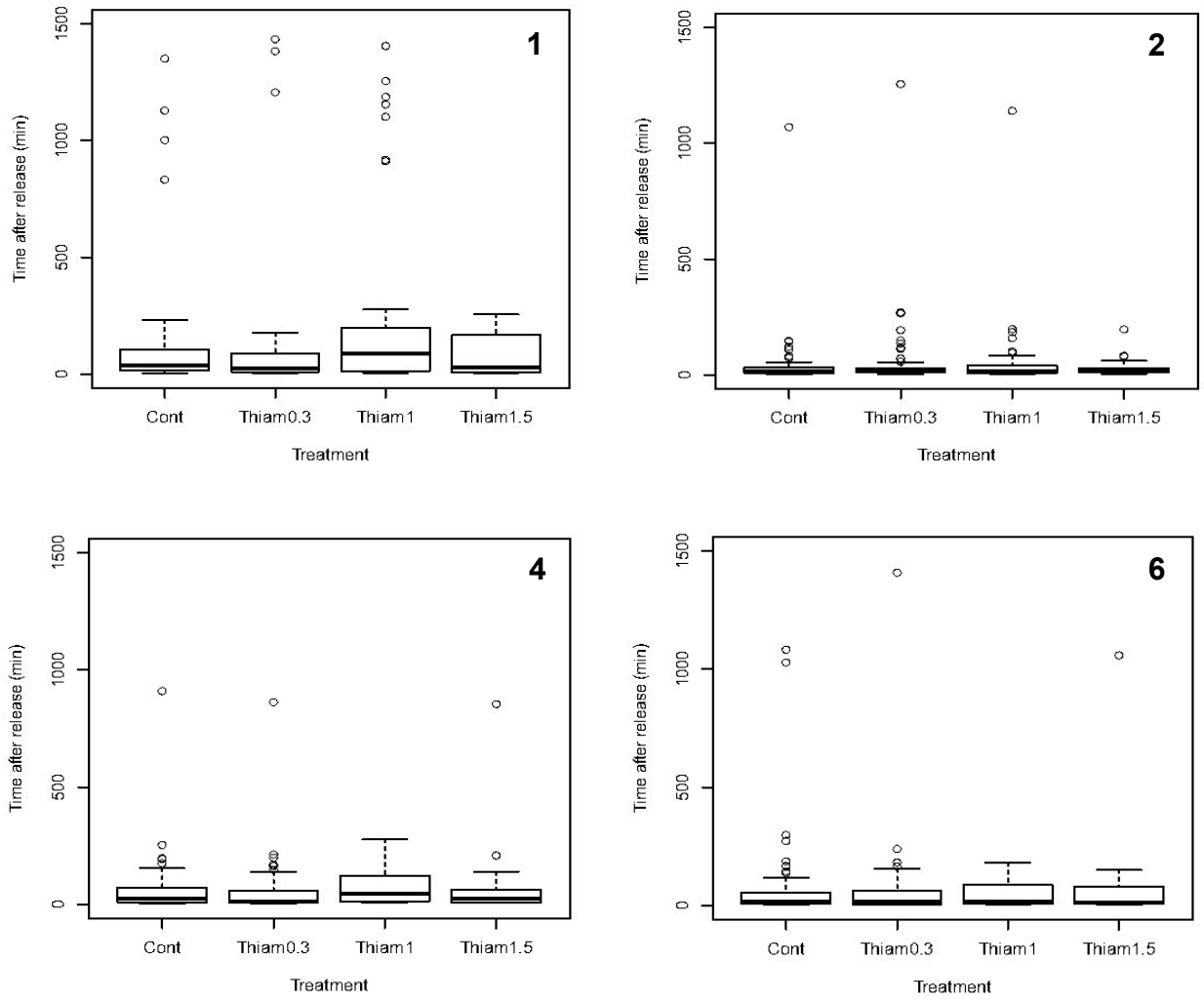
For all the labs in 2018, homing duration didn't significantly differ between groups of control bees and groups of bees exposed to 0.33 ng per bee of thiamethoxam (Kruskal-Wallis tests followed by Mann-Whitney tests; $P > 0.05$; Table 19 and Figure 13). For 3 labs out of 5, homing duration was significantly longer for the bees exposed to the highest doses (1 or 1.5 ng per bee) compared to control

bees or bees exposed to the lowest dose (0.33 ng per bee) (Kruskal-Wallis tests followed by Mann-Whitney tests; $P < 0.05$; Table 19 and Figure 13).

Table 19: Median homing duration for the ring test 2018 (three valid test runs pooled)

		Control	Thiam. 0.33 ng/bee	Thiam. 1 ng/bee	Thiam. 1.5 ng/bee
Lab 1	**Median homing duration in min (24 h after release)[1]**	**36.58 (ab)**	**24.61 (a)**	**88.64 (b)**	**29.57 (ab)**
Lab 2		14.80 (a)	18.62 (a)	16.77 (a)	16.43 (a)
Lab 4		**23.88 (ab)**	**13.82 (a)**	**46.28 (b)**	**23.67 (ab)**
Lab 6		15.59 (a)	15.55 (a)	16.87 (a)	13.72 (a)
Lab 8		**43.62 (ab)**	**42.07 (b)**	**33.19 (b)**	**80.26 (a)**

[1]For homing duration, Kruskal-Wallis tests were performed. When a significant difference was found ($P < 0.05$), pairwise comparisons were performed using Mann-Whitney tests and Bonferroni P value adjustment method. Same letters indicate no significant differences.



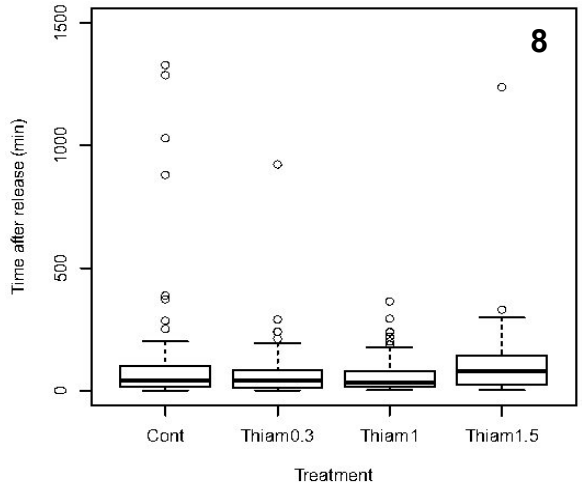


Figure 13: Homing duration of groups of foragers 24 hours after release in 2018 (three valid test runs pooled).

For all the labs in 2019, homing duration didn't significantly differ between groups of control bees and groups of bees exposed to thiamethoxam (Kruskal-Wallis tests followed by Mann-Whitney tests; $P > 0.05$; Table 20 and Figure 14).

Table 20: Median homing duration for the ring test 2019 (two to three test runs pooled)

		Control	Thiam. 0.33 ng/bee	Thiam. 1 ng/bee	Thiam. 1.5 ng/bee
Lab 1	**Median homing duration in min (24 h after release)[1]**	37.48 (a)	31.47 (a)	42.57 (a)	31.90 (a)
Lab 2		23.37 (a)	14.19 (a)	20.33 (a)	19.23 (a)
Lab 3		18.73 (a)	25.45 (a)	26.25 (a)	35.58 (a)
Lab 4		17.60 (a)	16.31 (a)	15.28 (a)	14.27 (a)
Lab 5		13.37 (a)	12.92 (a)	13.96 (a)	12.50 (a)
Lab 6		17.53 (a)	18.35 (a)	19.17 (a)	60.75 (a)

[1]For homing duration, Kruskal-Wallis tests were performed. When a significant difference was found ($P < 0.05$), pairwise comparisons were performed using Mann-Whitney tests and Bonferroni P value adjustment method. Same letters indicate no significant differences.

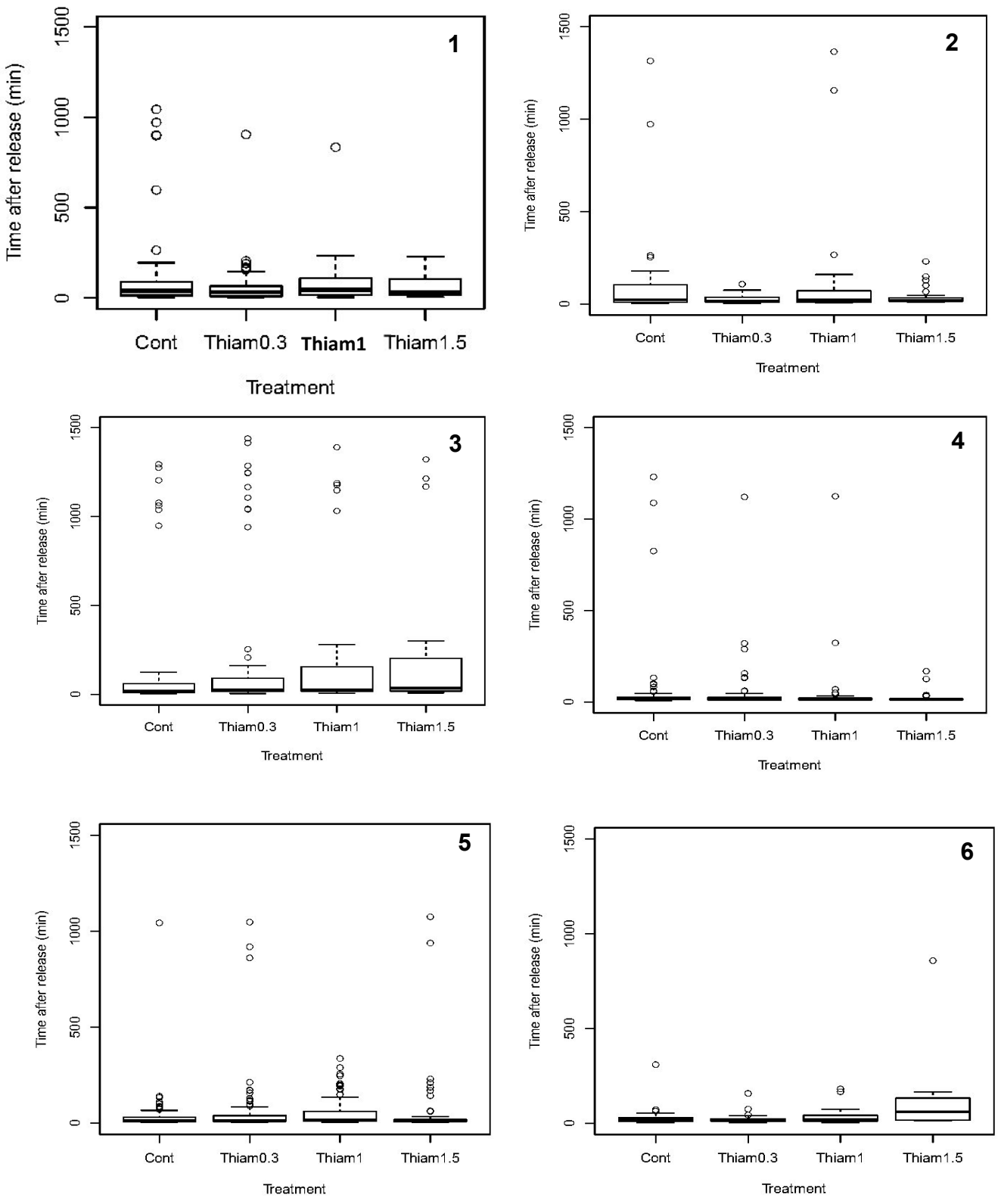


Figure 14: Homing duration of groups of foragers 24 hours after release in 2019 (two to three valid test runs pooled).

Details of statistical analyses for homing duration of each laboratory are presented in **Appendix 7**.

5.7 Variability of homing performances

Homing results, especially in exposed bees, may be modulated by different factors linked to environmental conditions (temperature and landscape, Henry et al. 2014) or health status of the colony, particularly Varroa (Monchanin et al. 2019) as homing performances are measured under field conditions.

Analyses were performed considering valid test runs only (**17 in 2018 and 16 in 2019**) and real doses to which the bees were exposed. The additional explanatory variables were punctual temperature at the release time, number of landmarks that the bees can cross during the travel back to the colony (indicator of landscape complexity), and number of Varroa per 100 bees (see **part 4.11**).

From the ring test data 2018, Generalized Linear Mixed Effects Models (GLMMs) showed no significant effect of environmental factors (temperature and landscape) alone or in interaction with treatment on homing performance of the bees (Table 21). But Varroa had a significant negative effect alone or in interaction with treatment. Results are illustrated in the Figures 15 and 16. Figure 15 is performed from raw data of valid test runs. Figure 16 is the model prediction of the reference item dose-response function of homing failure probability computed for different combinations of Varroa pressure. Results point to an aggravation of the homing failure of exposed bees with an increase of Varroa infestation of the colonies.

Table 21: Summary of the generalized linear mixed effect models (GLMM) performed on valid test runs to assess the effect of thiamethoxam dose, Varroa, temperature and landscape parameters as well as their interactions on honeybee homing success in 2018*.

GLMM Model parameter	Multimodel averaged estimate ± s.e.	Z	P-value
Intercept	**1.482 ± 0.603**	**2.457**	**< 0.05**
Dose	**-1.590 ± 0.610**	**2.605**	**< 0.01**
Landscape	0.905 ± 0.831	1.089	0.276
Varroa	**-0.970 ± 0.486**	**1.997**	**< 0.05**
Temperature	0.400 ± 0.639	0.626	0.531
Dose x Landscape	-1.213 ± 0.761	1.594	0.111
Dose x Temperature	-0.905 ± 0.693	1.305	0.192
Dose x Varroa	**-1.410 ± 0.490**	**2.876**	**< 0.01**

* Data associated with the two extreme real doses values (outliers, see **part 5.4**) were excluded from the analyses
s.e: Standard error
Z: Test statistic to assess if variables have a significant effect on homing performance

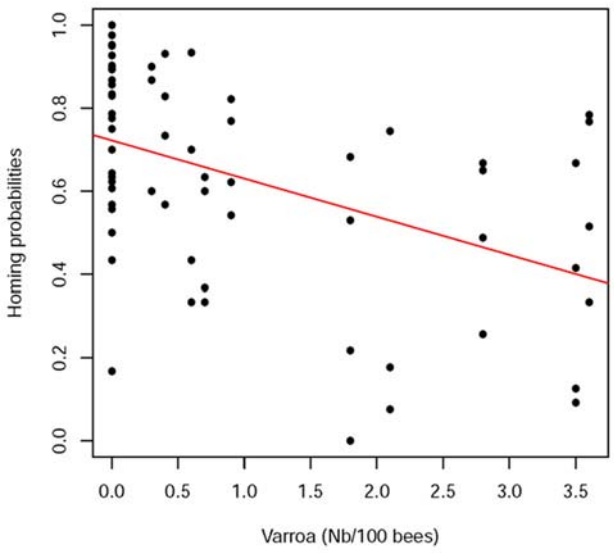


Figure 15: Relationships between homing probabilities of the foragers 24 hours after release and Varroa infestation of the colony (Nb of varroa per 100 bees). A red regression line was added.

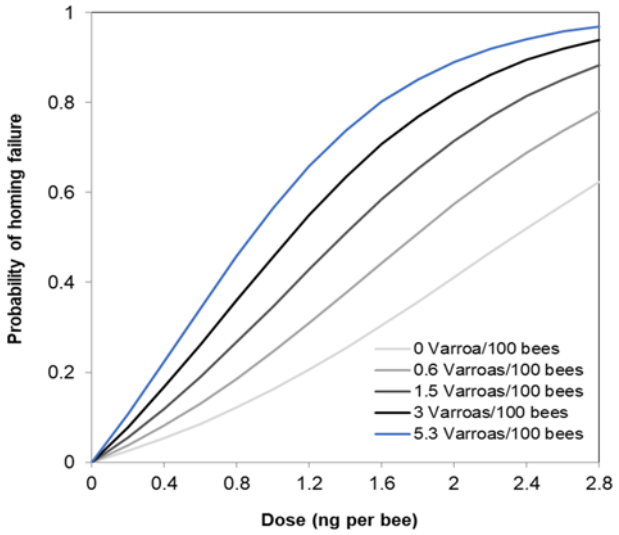


Figure 16: Model prediction of the referent item dose-response function of homing failure probability. The predicted dose-response curves were computed for different combinations of Varroa pressure ranging from no to more than 5 Varroas per 100 bees.

In 2019, GLMMs showed again no significant effect of temperature and landscape alone or in interaction with treatment on homing performance of the bees (Table 22). Varroa had a negative but not significant effect as a whole. However, when considering Varroa in interaction with treatment, a significant and positive effect was found (Table 22 and Figure 17). In 2019, the main majority of colonies tested had low or null Varroa infestation (**Appendix 8**). Only Lab 4 had highest varroa infestation (more than 5 varroas per 100 bees). But homing performances were not affected, especially in exposed bees, compared to the other labs. For the second test run of lab 4, we note high homing performances for bees exposed to the highest doses too (part 5.2, Table 11).

Table 22: Summary of the generalized linear mixed effect models (GLMM) performed on valid test runs to assess the effect of thiamethoxam dose, Varroa, temperature and landscape parameters as well as their interactions on honeybee homing success in 2019.

GLMM Model parameter	Multimodel averaged estimate ± s.e.	Z	P-value
Intercept	1.994 ± 0.395	**5.049**	**< 0.0001**
Dose	**-2.903 ± 0.298**	**9.755**	**< 0.0001**
Landscape	-0.103 ± 0.673	0.154	0.878
Varroa	-0.823 ± 0.714	1.152	0.249
Temperature	0.274 ± 0.950	0.288	0.774
Dose x Landscape	0.122 ± 0.492	0.248	0.804
Dose x Temperature	-0.429 ± 0.784	0.547	0.584
Dose x Varroa	**1.116 ± 0.449**	**2.486**	**< 0.05***

*P value = 0.013
s.e: standard error
Z: _Test statistic to assess if variables have a significant effect on homing performance

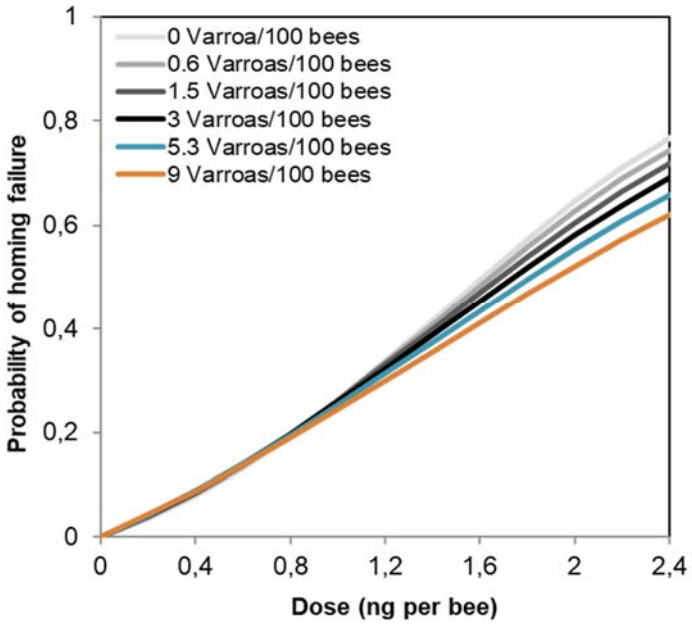


Figure 16: Model prediction of the reference item dose-response function of homing failure probability. The predicted dose-response curves were computed for different combinations of Varroa pressure ranging from no to 9 Varroas per 100 bees.

Varroa is one factor that may modulate homing performances especially in exposed bees. The parasite must be controlled as far as possible. In 2018, accepted valid test runs (n= 17) are not impacted with a varroa infestation at least equal or below to 4 and 5 varroas per 100 bees (Table 23). It also has to be noted that 2 invalid test runs (homing performances in control bees < 60%) performed in late August/beginning of September had a varroa pressure above 6 varroas per 100 bees (6.7 and 8.1 varroas per 100 bees, see annex 8 for 2018). Then, considering that the homing flight test may be performed from April to September (according to weather conditions and availability of blooming crops) and that varroa pressure may evolve during this time with the colony development, an acceptable infestation threshold of the colonies for the test could be **< 5 varroas per 100 bees**.

Table 23: Number of accepted and non-accepted valid test runs when varroa infestation of the colonies were less or equal to 3, 4 or 5 varroas per 100 bees

	≤ 3 varroas/100 bees	**≤ 4 varroas/100 bees**	**≤ 5 varroas/100 bees**
Accepted test runs	15	17	17
Not accepted test runs	2	0	0

This threshold of < **5 varroas per 100 bees** can be compared to informations found in literature. The most cited economic threshold warning for mortality risk of the colony and decreasing honey production ranged from 3 200 to 4 200 varroas per colony (Delaplane et al. 1999). Then, considering an average of 3700 varroas per colony, this is in accordance to a maximum of 4 to 5 varroas per 100 bees according to honeybee population size (http://extension.msstate.edu/sites/default/files/publications/publications/p2826_web.pdf; Table 1). In France, above 5 varroas per 100 bees, honey production from lavender crop can decrease to an average of 6.5 kg less per colony compared to colonies with less than 3 varroas per 100 bees. (Kretzschmar et al. 2016).

Varroa and Landscape data obtained in 2018 and 2019 are proposed in **Appendix 8**. Details of the GLMMs results are presented in **Appendix 9**.

5.8 Critical points with the homing flight method

From the questionnaire 2018, one objective was to try to identify possible remaining problems with the homing flight method, especially for labs that encounter difficulties to perform the test as a whole.

Problems	**Protocol 2018**	**Improvement**
Use of cold blocks directly with collected bees to transport them to the laboratory (2 labs in 2018)	No mention of cold blocks	Impact on the bees' maintenance during lab phase ➔ No use of cold block mentioned in the protocol 2019

Problems	Protocol 2018	Improvement
Collection of the foragers before coloring and release 1) Collection of foragers only going out the hive (1 lab in 2018 and 2019) 2) Collection of foragers only carrying nectar when entering the hive (1 lab in 2018)	All types of foragers carrying pellets of pollen or not (nectar or pollen)	Need to precise the type of foragers collected: 1) Capture of foragers entering the hive only because foraging trip is performed and bees are so expected to come back quicker to the hive after coloration (pink powder) and first release 2) Capture as far as possible of pollen collectors with expected low stomach content i) for better consumption and homogenization among bees via trophallaxis during exposure phase; ii) to prevent possible dilution that may occur when only nectar foragers with expected higher stomach content are collected (R&D results of 2 labs in 2018). ➔ Focus on pollen collectors was mentioned in the protocol 2019
Difficulties to label all the bees with RFID tags in 1h30 (1 lab in 2018 and 2019) More sucrose solution may be needed during exposure phase when the majority or all foragers are pollen collectors (Results of 1 lab in 2019)	Decrease of the labelling phase from 2h00 to 1h30 for the maintenance of the bees Not discussed for the protocol 2018; discussed after the ring test 2019	Labelling the bees in two hours may be risky (e.g; weak bees before the exposure phase) Training with the method, especially for the tricky phase of labelling is important For the draft TG, a list of conditions to increase the performance of successful homing test will be proposed (e.g. training with the method, especially labelling; exposure with 20 to 40* µl per bee of sucrose solution according to the bees'needs) *especially when only pollen foragers are used and according to the requirement of the bees.
Homing results may be different according to the mode of exposure: individual vs collective (10 bees feeding scheme)	From 2015, bees are exposed collectively as no differences were found between individual *vs* collective exposure (see Results of the First ring test 2015) but other authors showed more variability in homing performances with the collective exposure (Jeker & Grossar, 2020)	Collective exposure (10 bees feeding scheme) is kept for the homing flight test, for following reasons: - Differences between individual and collective exposure are not always observed, - Individual exposure is more time-consuming (caging, check of syrup consumption, release in field), -More people would be asked for the bees ' manipulation (e.g. at least one to two persons more for the labelling phase).

CONCLUSION

As a whole, the ring test results showed that the homing flight test matches different points for validation:
- **Feasibility**: a great majority of the labs could conduct the test with success (73 % of valid tests out of 41 tests performed over the 5 ring test years[2])
- **Sensitivity**: to detect of effects of sublethal doses of thiamethoxam on homing performances of foragers compared to control bees (77 % on 30 valid tests over the 5 ring test years)
- **Results reproducibility**: a majority of labs established the test endpoint (NOED in ng per bee) (77 % on 30 tests performed with success over the 5 ring test years; all the labs with successful tests determined a NOED in 2018 and 2019)

Validity criteria:
For each test run, mortality of the bees after exposure and before release met the validity criterion of ≤ 15% as a whole for control but also for exposed bees. The second validity criterion, the minimum and acceptable homing performances in control bees, showed to vary between 60 and 70 % according to the ring test results. Best homing success in control bees were obtained in 2017 but bees were fed *ad libitum* and results variability increased in exposed bees because of the dilution of the remaining stomach content. The last ring test year (2019) is a compromise for the NOED determination and for the homing performances of control bees. In 2019, nearly 70 % of the valid test runs met a minimum and acceptable homing success in control bees of ≥ 60 %. But when increasing the minimum and acceptable control rate to ≥ 70%, similar results were obtained with 65 % of valid test runs.

Comparison of the ring test results with "natural" foragers' loss under field condition is important to bring supplementary data (F. Requier, com. pers.). R&D study presented in this report showed that foragers' survival mainly vary between 70 and 100 %. Then, this study would support a maximum daily foragers' loss of 30 %. This is comparable to the minimum and acceptable level of 30 to 40 % of homing failure for the control bees in the homing test.

Factors of variability:
Environmental factors such as temperature and landscape, (number of landmarks) and Varroa were considered alone or in interaction with treatment to assess their possible effect in homing results variabilities, especially for exposed bees. No significant effect of temperature or landscape were found from the ring test data 2018 and 2019. But Varroa showed significant negative effects alone or in interaction with treatment in 2018. Varroa may modulate homing performances especially in exposed bees. As far as possible, colonies with low Varroa infestation are recommended to perform the test (**< 5 varroas per 100 bees** considering results 2018 and literature recommendations).

[2] We considered tests with 2 to 3 valid runs (homing success of control bees ≥ 60 %)

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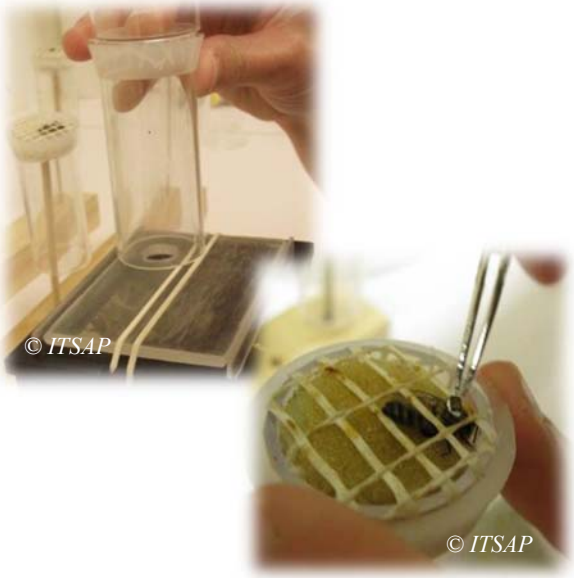
APPENDIX 1

Capture of the pink powdered bees at the hive entrance after release



Transfer to a holding cage and labelling with a RFID tag

Exposure phase per small group of 10 bees

Example of a RFID system for the homing flight ring test

APPENDIX 2

Protocol to control performance of the RFID system

- 6 « test » tags glued onto small plastic or wooden sticks ➔ UIDs of the tags first recorded
- Each tag is passed five times through each of the four readers ➔ 20 readings per tag expected and a total of 120 readings expected for the 6 test tags
- Tested tag must be read at least one time each time it passes through a reader
- Reading rates (%) is calculated as recorded data on expected data (120 readings)

The acceptance criteria for the performance of the RFIS system was that **at least 95% of the crossing of the tags should be recorded**.

Reading rate control of the RFID system 2018

Laboratory	Date	Total number of reading	Reading rate (%)
Lab 1	28/05/2018	120	100
Lab 2	09/07/2018	120	98.33
Lab 3	25/07/2018	120	100
Lab 4	29/06/2018	120	99.17
Lab 5	02/07/2018	120	99.17
Lab 6	13/08/2018	120	100
Lab 7	24/08/2018	120	97.50
Lab 8	29/05/2018	120	100

Reading rate control of the RFID system 2019

Laboratory	Date	Total number of reading	Reading rate (%)
Lab 1	13/05/2019	120	99.17
Lab 2	18/04/2019	120	98.33
Lab 3	03/06/2019	120	100
Lab 4	08/07/2019	120	100
Lab 5	24/04/2019	200	98.00
Lab 6	26/06/2019	120	98.33
Lab 7	20/06/2019	120	98.33
Lab 8	12/07/2019	6	100

APPENDIX 3

2018 and 2019

CLUZEAU INFO LABO
33-35 Rue Jean-Louis Faure, BP 88
33220 SAINTE-FOY-LA-GRANDE
☎ : 05 57 46 00 44

Dr. Ehrenstorfer
Reference Materials for Residue Analysis

Certificate of Analysis

ISO Guide 34 Reference Material

Product Identification

Articel Code:	DRE-C17453000
Artikel Name:	Thiamethoxam
Formula:	C8H10ClN5O3S
Mol. Weight:	291.71
CAS No.:	153719-23-4

Lot Number:	G133046
Expiry Date:	16.01.2021
Storage Temperature:	20°C ± 4°C

Storage and handling: The RM should be stored in the original sealed bottle at the temperatur given above. After use the bottle should be tightly closed and protected from moisture and light. The expiry date is valid for original closed bottles under recommended storage conditions only.

Purity	99.69%
Expanded Uncertainty U=	0.30%

The uncertainty of this standard is calculated in accordance with the ISO Guide 34 and EURACHEM/CITAC Guide - Quantifying Uncertainty in Analytical Measurement, Second Edition. The Expanded uncertainty is U = u(RM) x k, where k i the coverage factor at the 95% confidence Level (k=2). The expanded uncertainty U is bades on the combination of the uncertainties associated with each individual operation involved in the analysis of the product. $U(RM) = \sqrt{u(char)^2 + u(bb)^2 + u(lts)^2 + u(sts)^2}$; u(char) is the uncertainty of purity determination; u(bb) uncertainty of homogeneity test; u(lts) is uncertainty of stability test long-term; u(sts) is uncertainty of stability test short-term. u(lts) and u(sts) are not included in the calculation as the stability statement is based on real evidence opposed to simulation.
Minimum sample: 1 mg is recommended as the minimal sample amount. If less material is used, it is recommended to increase the certified uncertainty by a factor of two for half sample and a factor of four for a quarter of sample
Intended use: Use this RM as calibrant for chromatography or any other analytical technique.

Analytical Data

Traceability of chromatography: To the International System of Unity (SI).

Intrument:	HPLC/DAD		Method Details
Detection:	DAD		Acetonitrile:Water 4:1
Column:	ReproSil 100 C18 5 µm 250 x 3 mm		
Inj.-Vol.:	10	µl	
Flow:	1	ml/min	
Ret.Time:	1.15	min	

Comment
Traceability: The balances used are calibrated with weights traceable to the national standards (DKD).
Calibrated Class A glassware is used for volumetric measurements.
Certificate Revision 1
Water Content: 0.26% (g/g) by Karl-Fischer-Titration (U(exp) = 0.22% (g/g)).
Identity: EA, NMR, RT, IR, UV

Certified on: 18.05.2017
Certified by: N. Müller

Authorized copy
from the original
19. MAI 2017
Sign.:

The Laboratory LGC Labor GmbH is accreditated by DAkkS as indicated by the Accreditations Number D-RM-19883-01 & D-PL-19883-01 has shown competence based on ISO Guide 34:2009 with relevant parts of DIN EN ISO/IEC 17025:2005 for production of certified reference materials in form of organic pure substances and in form of single and multi-component solutions of organic pure substances.

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Phone +49 821 906080 - Fax +49 821 9060888 - augsburg.inquiry@lgcgroup.com
The warranty for this product is limited to the purchasing price of this product

Data File L:\GERÄTE BACKUP\DAD3\2017\2017KW02\120117-2 2017-01-12 13-08-35\17453000-1.D

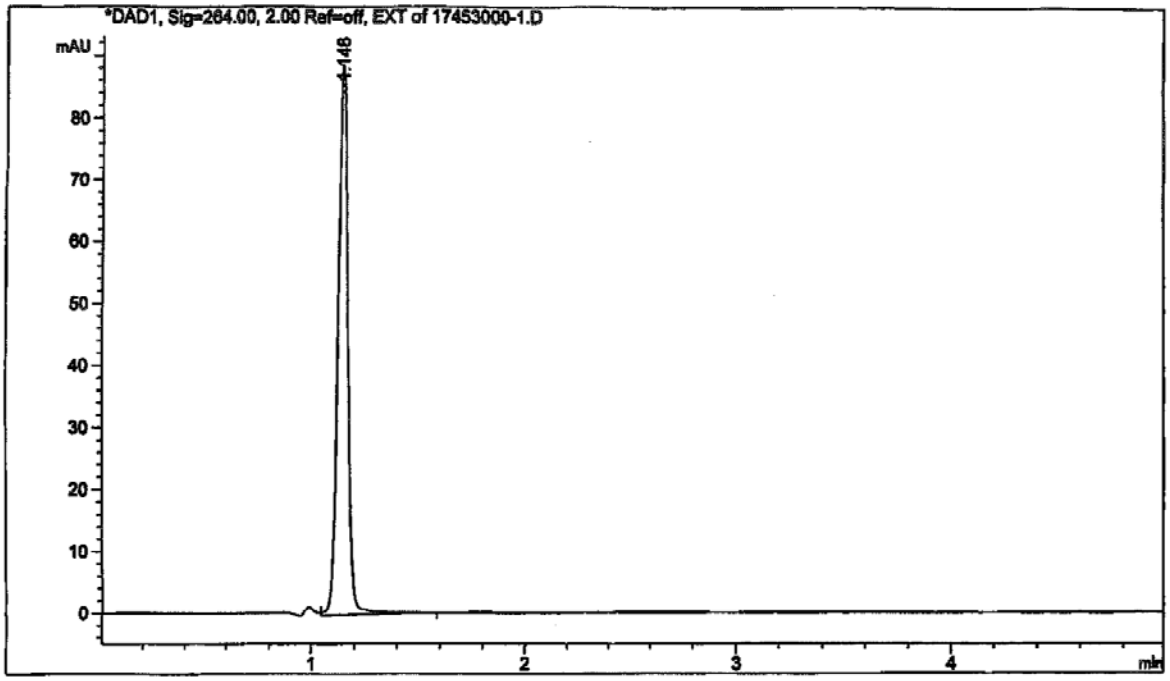
13.01.17 HQ

Acq. Operator : DAD3_Admin
Acq. Instrument : DAD3
Injection Date : 12.01.2017 22:00:40

Seq. Line : 36
Location : 82
Inj : 1
Inj Volume : 10.000 µl

Acq. Method : C:\Chem32\1\DATA\2017KW02\120117-2 2017-01-12 13-08-35\41K.M
Last changed : 08.11.2016 07:28:53 by DAD3_Admin
Analysis Method : L:\GERÄTE BACKUP\DAD4\METHODS\41K.M
Last changed : 29.10.2015 10:40:19 by SYSTEM
Method Info : Acetonitrile : Water 4:1

Sample Info : Thiamethoxam



Area Percent Report

Sorted By : Retention Time
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1, Sig=264.00, 2.00 Ref=off, EXT
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	1.146	1	VB	280.75775	89.04220	100.0000
Totals :				280.75775	89.04220	

*** End of Report ***

APPENDIX 4

Preparation of the item, test solutions and test feeding solutions

- ⇨ 20 µl of sucrose solution (30 % w/v) per bee containing 0.1% of acetone is considered
- ⇨ Test item doses: 0.33, 1 and 1.5 ng per bee

1- Preparation of the stock solution (S)

***1.5 ng test item in 0.02 µl acetone => 75 ng/µl or 75 µg/ml*

Preparation of a one hundred time more concentrated « S »:

75 x 100 = 7 500 µg/ml or 7.5 mg/ml

To prepare « S » => 15 mg of thiamethoxam is weighed and 2 ml of acetone is added

2- Preparation of a 1.5 ng per bee test solution (S1)

Dilution 1/100: solution « S1 » at 75 µg/ml

10 ml as a final acetone volume is considered.

Preparation:

Ci xVi = Cf xVf => 7500 µg/ml x Vi = 75 µg/ml x 10

Vi = 0.1 ml => 100 µl of S is sampled and 9.9 ml of acetone is added

3- Preparation of a 1 ng per bee test solution (S2)

Dilution 2/3: solution S2 at 50 µg/ml; 3 ml as a final acetone volume is considered

Preparation :

Ci xVi = Cf xVf => 75 µg/ml x Vi = 50 µg/ml x 3

Vi = 2 ml => 2 ml of S1 is sampled and 1 ml of acetone is added

4- Preparation of a 0.33 ng per bee test solution (S3)

Dilution 1/3: solution S3 at 16.667 µg/ml; 3 ml as a final acetone volume is considered

Preparation : 1 ml of S2 is sampled and 2 ml of acetone is added

5- Test feeding solutions

General preparation: 15 g of sugar in 50 ml of demineralized water (30 % w/v)

Four samples of 10 ml of this sucrose solution are prepared for the 3 tested and control treatments.

Test feeding solutions are prepared in 10 ml of sucrose solution:

Treatment	Test solution sample in µl	Sucrose solution (30% w/v) in ml
Control (acetone)	10 µl acetone	10
Thiamethoxam 1 ng	10 µl S1	10
Thiamethoxam 0.33 ng	10 µl S2	10
Thiamethoxam 0.11 ng	10 µl S3	10

APPENDIX 5

Percentage of pollen foragers captured at the hive entrance for the homing flight ring test 2019

Lab	Run	Pollen foragers (%)
1	1	60
	2	45
	3	40
2	1	90
	2	80
	3	50
3	1	100
	2	100
	3	100
4	1	70
	2	
5	1	18.5
	2	34
	3	28
6	1	90
	2	90
	3	70
7	1	Capture of bees going out the hive
	2	Capture of bees going out the hive
	3	Capture of bees going out the hive
8	1	Not determined Capture of all type of foragers entering the hive
	2	Not determined Capture of all type of foragers entering the hive
	3	Not determined Capture of all type of foragers entering the hive

APPENDIX 6

A) Punctual weather conditions (temperature, hygrometry, cloud layer and wind strength) at the time of the bees release during the homing flight ring test 2018

Lab	Run	Mean temperature (°C)	Mean hygrometry (%)	Cloud layer	Wind strength
1	1	21.5	40	Low	Low
	2	22.4	45	Average	Null
	3	27.2	42	Low	Low
2	1	32.2	37.3	Null	Average
	2	33.5	35.1	High	Null
	3	23.2	46.5	High	Low
3	1	35.6	23.7	Null	Null
	2	31.7	33.5	Null	Null
	3	27.2	30.6	Null	Null
4	1	35.3	25	Null	Null
	2	34.6	10	Null	Null
	3	39.2	21	Null	Null
5	1	29	53	Low	Low
	2	33	42	Low	Low
	3	33	39	Low	Low
6	1	22.1	62	High	Low
	2	27.2	51	Average	Null
	3	28	22	Null	Null
7	1	26.2	41	Low	Low
	2	-	-	Low	Low
	3	31	-	Low	Low
8	1	30.4	48.6	Low	Low
	2	25.5	46.4	Low	Low
	3	28.5	43.6	Low	Low

B) Punctual weather conditions (temperature, hygrometry, cloud layer and wind strength) at the time of the bees release during the homing flight ring test 2019

Lab	Run	Mean temperature (°C)	Mean hygrometry (%)	Cloud layer	Wind strength
1	1	31	19	Null	Null
	2	32.8	15	Low	Low
	3	38.5	16	Null	Null
2	1	26.4	30.2	Low	Null
	2	30.9	45.6	Low	Low
	3	30.0	24.9	Average	Low
3	1	26	42	Low	Low
	2	24	67	Average	Average
	3	26	40	Low	Low
4	1	24.2	49	Null	Null
	2	29.4	42.1	Low	Null
5	1	27.7	43	Low	Null
	2	24.4	43	Average	Low
	3	26.7	50	High	Null
6	1	23	72.5	Low	Low
	2	25	55	Average	Average
	3	26	50	Average	Low
7	1	34	40	Null	Null
	2	34	51	Null	Null
	3	34	33	Null	Null
8	1	28.9	27	Low	Null
	2	27.3	43	Average	Low
	3	27	52	Low	Null

APPENDIX 7

Detail of the statistical analysis performed on homing success and homing duration 24 hours after the release of the bees

2018 – HOMING SUCCESS

Lab 1

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)

4-sample test for equality of proportions without continuity correction
data:  tab_cont

X-squared = 87.716, df = 3, p-value < 2.2e-16
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3    prop 4
0.1860465 0.2093023 0.4022989 0.8148148
```

***Multiple comparisons after Chi² (without P value adjustment)**

```
> pairwise.prop.test(tab_cont, p.adj = "none")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.8482	-	-
Thiam1	**0.0032**	**0.0096**	-
Thiam1.5	**1.6e-15**	**1.8e-14**	**1.2e-07**

***P value adjustment method : Bonferroni**

```
> pairwise.prop.test(tab_cont, p.adj = "bonferroni")
 Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.000	-	-
Thiam1	**0.019**	0.058	-
Thiam1.5	**9.7e-15**	**1.1e-13**	**7.0e-07**

Lab 2

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)

4-sample test for equality of proportions without continuity
       correction
data:  tab_cont
```

X-squared = 33.219, df = 3, p-value = 2.895e-07

```
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3    prop 4
0.2022472 0.2584270 0.4888889 0.5694444
```

***Multiple comparisons after Chi² (without P value adjustment)**

```
> pairwise.prop.test(tab_cont, p.adj = "none")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.47643	-	-
Thiam1	**0.00011**	**0.00244**	-
Thiam1.5	**3.4e-06**	**0.00012**	0.38875

***P value adjustment method : Bonferroni**

```
> pairwise.prop.test(tab_cont, p.adj = "bonferroni")
  Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.00000	-	-
Thiam1	**0.00065**	**0.01462**	-
Thiam1.5	**2.1e-05**	**0.00072**	1.00000

Lab 4

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)

4-sample test for equality of proportions without continuity
       correction
data:  tab_cont
```

X-squared = 19.415, df = 3, p-value = 0.0002244

```
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3    prop 4
0.2921348 0.3563218 0.4871795 0.6052632
```

***Multiple comparisons after Chi² test (without P value adjustment)**

> pairwise.prop.test(tab_cont, p.adj = "none")

Pairwise comparisons using Pairwise comparison of proportions

data: tab_cont

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.4540	-	-
Thiam1	**0.0152**	0.1228	-
Thiam1.5	**0.0001**	**0.0025**	0.1904

***P value adjustment method: Bonferroni**

> pairwise.prop.test(tab_cont, p.adj = "bonferroni")

Pairwise comparisons using Pairwise comparison of proportions

data: tab_cont

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.00000	-	-
Thiam1	0.09133	0.73660	-
Thiam1.5	**0.00061**	**0.01524**	1.00000

Lab 6

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
```

> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)

4-sample test for equality of proportions without continuity correction

data: tab_cont

X-squared = 79.931, df = 3, p-value < 2.2e-16

alternative hypothesis: two.sided

sample estimates:

prop 1	prop 2	prop 3	prop 4
0.2758621	0.2777778	0.6491228	0.7452830

***Multiple comparisons after Chi² test (without P value adjustment)**

> pairwise.prop.test(tab_cont, p.adj = "none")

Pairwise comparisons using Pairwise comparison of proportions

data: tab_cont

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.00	-	-
Thiam1	**2.9e-08**	**6.4e-08**	-
Thiam1.5	**7.2e-12**	**2.0e-11**	0.16

***P value adjustment method: Bonferroni**

```
> pairwise.prop.test(tab_cont, p.adj = "bonferroni")
  Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.00	-	-
Thiam1	**1.8e-07**	**3.8e-07**	-
Thiam1.5	**4.3e-11**	**1.2e-10**	0.97

Lab 8

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)
4-sample test for equality of proportions without continuity
      correction
data:  tab_cont
```

X-squared = 29.882, df = 3, p-value = 1.461e-06

```
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3    prop 4
0.09016393 0.05128205 0.07200000 0.25423729
```

***Multiple comparisons after Chi² test (without P value adjustment)**

```
> pairwise.prop.test(tab_cont, p.adj = "none")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.35898	-	-
Thiam1	0.77187	0.68826	-
Thiam1.5	**0.00135**	**3.5e-05**	**0.00022**

***P value adjustment method: Bonferroni**

```
> pairwise.prop.test(tab_cont, p.adj = "bonferroni")
  Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.00000	-	-
Thiam1	1.00000	1.00000	-
Thiam1.5	**0.00811**	**0.00021**	**0.00133**

2019 – HOMING SUCCESS

Lab 1

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)
4-sample test for equality of proportions without continuity correction
data:  tab_cont

X-squared = 71.982, df = 3, p-value = 1.606e-15
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3    prop 4
0.1904762 0.1647059 0.3000000 0.7215190
```

***Multiple comparisons after Chi² test (without p value adjustment)**

```
> pairwise.prop.test(tab_cont, p.adj = "none")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Con t	Thiam0.3	Thiam1
Thiam0.3	0.81	-	-
Thiam1	0.15	0.06	-
Thiam1.5	**2.8e-11**	**2.0e-12**	**2.5e-07**

***P value adjustment method : Bonferroni**

```
> pairwise.prop.test(tab_cont, p.adj = "bonferroni")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.00	-	-
Thiam1	0.88	0.36	-
Thiam1.5	**1.7e-10**	**1.2e-11**	**1.5e-06**

Lab 2

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)
4-sample test for equality of proportions without continuity correction
data:  tab_cont

X-squared = 31.633, df = 3, p-value = 6.255e-07
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3    prop 4
0.2808989 0.3636364 0.5333333 0.6750000
```

***Multiple comparisons after Chi² test (without p value adjustment)**

```
> pairwise.prop.test(tab_cont, p.adj = "none")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.30915	-	-
Thiam1	**0.00102**	**0.03360**	-
Thiam1.5	**6.6e-07**	**0.00011**	0.08451

***P value adjustment method: Bonferroni**

```
> pairwise.prop.test(tab_cont, p.adj = "bonferroni")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.00000	-	-
Thiam1	**0.00614**	0.20158	-
Thiam1.5	**4e-06**	**0.00063**	0.50705

Lab 3

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)
4-sample test for equality of proportions without continuity correction
data:  tab_cont
```

X-squared = 83.179, df = 3, p-value < 2.2e-16

```
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3    prop 4
0.1647059 0.1463415 0.4074074 0.7439024
```

***Multiple comparisons after Chi² test (without p value adjustment)**

```
> pairwise.prop.test(tab_cont, p.adj = "none")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.90942	-	-
Thiam1	**0.00098**	**0.00038**	-
Thiam1.5	**1.7e-13**	**4.6e-14**	**2.8e-05**

***P value adjustment method: Bonferroni**

```
> pairwise.prop.test(tab_cont, p.adj = "bonferroni")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.00000	-	-
Thiam1	**0.00586**	**0.00229**	-
Thiam1.5	**1.0e-12**	**2.8e-13**	**0.00017**

Lab 4

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)
4-sample test for equality of proportions without continuity correction
data:  tab_cont
```

X-squared = 18.881, df = 3, p-value = 0.0002893

```
alternative hypothesis: two.sided
sample estimates:
   prop 1    prop 2    prop 3    prop 4
0.2278481 0.2368421 0.4000000 0.5063291
```

***Multiple comparisons after Chi² test (without p value adjustment)**

```
> pairwise.prop.test(tab_cont, p.adj = "none")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.00000	-	-
Thiam1	**0.03306**	**0.04793**	-
Thiam1.5	**0.00053**	**0.00097**	0.24497

***P value adjustment method: Bonferroni**

```
> pairwise.prop.test(tab_cont, p.adj = "bonferroni")
Pairwise comparisons using Pairwise comparison of proportions
data:  tab_cont
```

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.0000	-	-
Thiam1	0.1984	0.2876	-
Thiam1.5	**0.0032**	**0.0058**	1.0000

Lab 5

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)
```

4-sample test for equality of proportions without continuity correction
data: tab_cont

X-squared = 42.454, df = 3, p-value = 3.214e-09
alternative hypothesis: two.sided
sample estimates:

prop 1	prop 2	prop 3	prop 4
0.06140351	0.09649123	0.26956522	0.35344828

***Multiple comparisons after Chi² test (without p value adjustment)**

```
>pairwise.prop.test(tab_cont, p.adj = "none")
```

Pairwise comparisons using Pairwise comparison of proportions
data: tab_cont

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.4613	-	-
Thiam1	**5.0e-05**	**0.0013**	-
Thiam1.5	**1.2e-07**	**6.8e-06**	0.2171

***P value adjustment method: Bonferroni**

```
>pairwise.prop.test(tab_cont, p.adj = "bonferroni")
```

Pairwise comparisons using Pairwise comparison of proportions
data: tab_cont

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.0000	-	-
Thiam1	**0.0003**	**0.0079**	-
Thiam1.5	**7.5e-07**	**4.1e-05**	1.0000

Lab 6

```
> tab_cont<-table(ab1$Treat,ab1$rfid_release_min)
> prop.test(tab_cont, alternative='two.sided',conf.level=.95,correct=FALSE)
```

4-sample test for equality of proportions without continuity correction

data: tab_cont
X-squared = 43.956, df = 3, p-value = 1.542e-09
alternative hypothesis: two.sided
sample estimates:

prop 1	prop 2	prop 3	prop 4
0.2711864	0.2881356	0.5833333	0.8076923

***Multiple comparisons after Chi² test (without p value adjustment)**

> pairwise.prop.test(tab_cont, p.adj = "none")
Pairwise comparisons using Pairwise comparison of proportions
data: tab_cont

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.0000	-	-
Thiam1	**0.0011**	**0.0022**	-
Thiam1.5	**4.9e-08**	**1.3e-07**	**0.0188**

***P value adjustment method : Bonferroni**

> pairwise.prop.test(tab_cont, p.adj = "bonferroni")
Pairwise comparisons using Pairwise comparison of proportions
data: tab_cont

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.0000	-	-
Thiam1	**0.0068**	**0.0132**	-
Thiam1.5	**2.9e-07**	**7.6e-07**	0.1125

2018 – HOMING DURATION

Lab 1

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 8.0622, df = 3, p-value = 0.04474

***Multiple comparisons with Mann-Whitney test (without p value adjustment)**

pairwise.wilcox.test(ab$rfid_release_min,ab$Treat,p.adjust.method="none")
Pairwise comparisons using Wilcoxon rank sum test
data: ab$rfid_release_min and ab$Treat

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.1464	-	-
Thiam1	0.0945	**0.0059**	-
Thiam1.5	0.8900	0.5501	0.2958

***P value adjustment method : Bonferroni**

> pairwise.wilcox.test(ab$rfid_release_min,ab$Treat,p.adjust.method="bonferroni")
Pairwise comparisons using Wilcoxon rank sum test
data: ab$rfid_release_min and ab$Treat

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.879	-	-
Thiam1	0.567	**0.036**	-
Thiam1.5	1.000	1.000	1.000

Lab 2

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 1.1436, df = 3, p-value = 0.7666

Lab 4

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 10.404, df = 3, p-value = 0.01542

***Multiple comparisons with Mann-Whitney test (without p value adjustment)**

pairwise.wilcox.test(ab$rfid_release_min,ab$Treat,p.adjust.method="none")
Pairwise comparisons using Wilcoxon rank sum test
data: ab$rfid_release_min and ab$Treat

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.140	-	-
Thiam1	0.042	**0.002**	-
Thiam1.5	0.879	0.156	0.111

***P value adjustment method : Bonferroni**

> pairwise.wilcox.test(ab$rfid_release_min,ab$Treat,p.adjust.method="bonferroni")
Pairwise comparisons using Wilcoxon rank sum test
data: ab$rfid_release_min and ab$Treat

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.842	-	-
Thiam1	0.250	**0.012**	-
Thiam1.5	1.000	0.937	0.665

Lab 6

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 2.9517, df = 3, p-value = 0.3991

Lab 8

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 13.566, df = 3, p-value = 0.003559

***Multiple comparisons with Mann-Whitney test (without p value adjustment)**

pairwise.wilcox.test(ab$rfid_release_min,ab$Treat,p.adjust.method="none")
Pairwise comparisons using Wilcoxon rank sum test
data: ab$rfid_release_min and ab$Treat

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.39792	-	-
Thiam1	0.50530	0.91868	-
Thiam1.5	**0.01275**	**0.00076**	**0.00170**

***P value adjustment method: Bonferroni**

> pairwise.wilcox.test(ab$rfid_release_min,ab$Treat,p.adjust.method="bonferroni")
Pairwise comparisons using Wilcoxon rank sum test
data: ab$rfid_release_min and ab$Treat

	Cont	Thiam0.3	Thiam1
Thiam0.3	1.0000	-	-
Thiam1	1.0000	1.0000	-
Thiam1.5	0.0765	**0.0046**	**0.0102**

2019 – HOMING DURATION

Lab 1

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 2.7563, df = 3, p-value = 0.4307

Lab 2

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 6.0721, df = 3, p-value = 0.1082

Lab 3

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 9.905, df = 3, p-value = 0.01939

***Multiple comparisons with Mann-Whitney test (without p value adjustment)**

pairwise.wilcox.test(ab$rfid_release_min,ab$Treat,p.adjust.method="none")
Pairwise comparisons using Wilcoxon rank sum test

data: ab$rfid_release_min and ab$Treat

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.051	-	-
Thiam1	**0.011**	0.411	-
Thiam1.5	**0.016**	0.241	0.473

***P value adjustment method: Bonferroni**

> pairwise.wilcox.test(ab$rfid_release_min,ab$Treat,p.adjust.method="bonferroni")
Pairwise comparisons using Wilcoxon rank sum test
data: ab$rfid_release_min and ab$Treat

	Cont	Thiam0.3	Thiam1
Thiam0.3	0.308	-	-
Thiam1	0.068	1.000	-
Thiam1.5	0.098	1.000	1.000

Lab 4

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 4.9276, df = 3, p-value = 0.1772

Lab 5

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 3.4626, df = 3, p-value = 0.3256

Lab 6

kruskal.test(ab$rfid_release_min~ab$Treat)
Kruskal-Wallis rank sum test
data: ab$rfid_release_min by ab$Treat
Kruskal-Wallis chi-squared = 5.9733, df = 3, p-value = 0.1129

APPENDIX 8

Assessment of Varroa infestation of the colonies for the ring test 2018 and 2019

A) 2018

Lab	Run	Sample date	Number of varroas per sample	Number of bees per sample	Number of varroas per 100 bees
1	1	14/06/2018	0	232,5	0,0
1	2	15/06/2018	0	234,2	0,0
1	3	18/06/2018	1	345,1	0,3
2	1	24/05/2018	2	335,7	0,6
2	2	31/08/2018	3	731,1	0,4
2	3	31/08/2018	2	295,5	0,7
3	1	27/07/2018	16	833,1	1,9
3	2	15/08/2018	3	672,3	0,4
3	3	24/08/2018	27	402,3	6,7
4	1	03/07/2018	3	346,4	0,9
4	2	10/07/2018	0	592,9	0,0
4	3	19/07/2018	19	537,9	3,5
5	1	26/06/2018	7	187,4	3,7
5	2	26/06/2018	4	217,9	1,8
5	**3**	26/06/2018	3	139,7	2,1
6	1	14/08/2018	12	431,9	2,8
6	2	21/08/2018	11	512,6	2,1
6	3	27/09/2018	10	279,6	3,6
7	1	27/08/2018	10	396,6	2,5
7	2	03/09/2018	18	222,3	8,1
7	3	14/09/2018	0	285,7	0,0
8	1	29/05/2018	0	356,9	0,0
8	2	04/06/2018	0	411,1	0,0
8	3	04/06/2018	0	423,4	0,0

B) 2019

Lab	Run	Sample date	Number of varroas per sample	Number of bees per sample	Number of varroas per 100 bees
1	1	13/06/2019	0	807,1	0,0
1	2	13/06/2019	0	564,3	0,0
1	3	13/06/2019	4	835,7	0,5
2	1	15/05/2019	0	278,6	0,0
2	2	15/05/2019	2	292,9	0,7
2	3	15/05/2019	0	335,7	0,0
3	1	16/06/2019	0	178,6	0,0
3	2	06/06/2019	0	178,6	0,0
3	3	12/06/2019	0	150,0	0,0
4	1	10/07/2019	62	814,3	7,6
4	2	17/07/2019	24	414,3	5,8
5	1	18/06/2019	0	500,0	0,0
5	2	21/06/2019	1	407,1	0,2
5	3	08/08/2019	7	321,4	2,2
6	1	27/06/2019	9	271,4	3,3
6	2	01/08/2019	12	350,0	3,4
6	3	08/08/2019	8	300,0	2,7
7	1	20/06/2019	0	207,1	0,0
7	2	20/06/2019	0	228,6	0,0
7	3	20/06/2019	0	157,1	0,0
8	1	05/08/2019	0	221,4	0,0
8	2	05/08/2019	0	464,3	0,0
8	3	05/08/2019	1	385,7	0,3

Landscape description (number of linears) for the ring test 2018 and 2019

A) 2018

Lab	Line	Hedge	River	Orchad	Building	Road	TOTAL
1	1	1	0	0	13	7	76
1	2	0	0	0	21	11	
1	3	0	0	0	14	9	
2	1	4	0	0	1	4	33
2	2	5	0	0	3	6	
2	3	6	0	0	1	3	
3	1	3	0	0	4	4	50
3	2	1	0	0	9	6	
3	3	1	0	0	16	6	
4	1	6	0	0	0	5	32
4	2	7	0	0	0	4	
4	3	7	0	0	2	1	
5	1	0	0	0	0	0	13
5	2	2	0	0	0	0	
5	3	4	0	0	0	0	
6	1	3	0	0	3	3	22
6	2	2	0	0	3	3	
6	3	2	0	0	1	2	
7	1	0	0	0	0	0	6
7	2	2	0	0	0	0	
7	3	4	0	0	0	0	
8	1	2	1	0	8	2	38
8	2	3	1	0	5	1	
8	3	3	0	0	9	3	

Hedge : Cypress, wooden hedge…

B) 2019

Lab	Line	Hedge	River	Orchad	Building	Road	TOTAL
1	1	6	0	0	0	5	32
1	2	7	0	0	0	4	
1	3	7	0	0	2	1	
2	1	4	0	0	1	4	33
2	2	5	0	0	3	6	
2	3	6	0	0	1	3	
3	1	1	0	0	13	7	76
3	2	0	0	0	21	11	
3	3	0	0	0	14	9	
4	1	3	0	0	4	4	52
4	2	1	0	0	9	6	
4	3	1	0	0	17	7	
5	1	5	0	0	2	3	23
5	2	2	0	0	2	3	
5	3	3	0	0	1	2	
6	1	1	0	0	0	3	21
6	2	3	0	0	1	3	
6	3	6	0	0	2	2	
7	1	5	0	0	2	4	43
7	2	4	0	0	5	3	
7	3	7	0	0	10	3	
8	1	0	0	0	1	4	19
8	2	2	0	0	1	6	
8	3	2	0	0	0	3	

Hedge : Cypress, wooden hedge…

APPENDIX 9

Detail of the generalized linear mixed effect models (GLMMs) performed on valid test runs to assess the effect of thiamethoxam dose, Varroa, temperature (punctual temperature at the release time) and landscape (number of linears) parameters as well as their interactions on honeybee homing success in 2018 and 2019

2018

Retour => Homing
Doser => Dose
Tempr => temperature
VarroaLg10r => Varroa Log 10 tranformed
Land Lg10 => Landscape Log 10 transformed

Step 1:

```
>succes2<glmer(Retour~Doser*Tempr+Doser*VarroaLg10r+Doser*LandLg10r+(1|Sitef)+(1|Sitef:
Hive), family=binomial, data=res1, na.action="na.fail")
> summary(succes2)

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: Retour ~ Doser * Tempr + Doser * VarroaLg10r + Doser * LandLg10r +
    (1 | Sitef) + (1 | Sitef:Hive)
   Data: res1

     AIC      BIC   logLik deviance df.resid
  2254.1   2310.6  -1117.1   2234.1     2072

Scaled residuals:
    Min      1Q  Median      3Q     Max
-5.0857 -0.7885  0.4227  0.6175  4.2456

Random effects:
 Groups     Name        Variance Std.Dev.
 Sitef:Hive (Intercept) 0.1369   0.3700
 Sitef      (Intercept) 0.2126   0.4611
Number of obs: 2082, groups:  Sitef:Hive, 17; Sitef, 7

Fixed effects:
              Estimate Std. Error z value Pr(>|z|)
(Intercept)     0.7861     0.6790   1.158  0.24701
Doser          -0.4952     0.6979  -0.710  0.47799
Tempr           0.7145     0.6364   1.123  0.26157
VarroaLg10r    -0.9453     0.4835  -1.955  0.05057 .
LandLg10r       1.2388     0.8304   1.492  0.13576
```

```
Doser:Tempr       -1.0818     0.6840  -1.582  0.11375
Doser:VarroaLg10r  -1.6023     0.4973  -3.222  0.00127 **
Doser:LandLg10r    -1.4844     0.7697  -1.929  0.05378 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
            (Intr) Doser  Tempr  VrrL10 LndL10 Dsr:Tm D:VL10
Doser       -0.365
Tempr       -0.550  0.226
VarroaLg10r -0.249  0.153 -0.190
LandLg10r   -0.847  0.287  0.260  0.103
Doser:Tempr  0.207 -0.624 -0.386  0.026 -0.094
Dsr:VrrLg10  0.158 -0.507  0.006 -0.368 -0.073  0.116
Dsr:LndLg10  0.326 -0.855 -0.123 -0.076 -0.354  0.320  0.295
```

Step 2:

```
> model.set <- dredge(succes2,rank="AIC")
Fixed term is "(Intercept)"
Warning messages:
1: In checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv,  :
  Model failed to converge with max|grad| = 0.00193754 (tol = 0.001, component 1)
2: In checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv,  :
  Model failed to converge with max|grad| = 0.00297695 (tol = 0.001, component 1)

> model.set
Global model call: glmer(formula = Retour ~ Doser * Tempr + Doser * VarroaLg10r +
    Doser * LandLg10r + (1 | Sitef) + (1 | Sitef:Hive), data = res1,
    family = binomial, na.action = "na.fail")
---
Model selection table
     (Int)     Dsr    LL1       Tmp     VL1 Dsr:LL1 Dsr:Tmp Dsr:VL1 df
74   1.8460 -1.9090                -1.0010                -1.345  6
92   1.2030 -1.1720 1.0070          -0.8762 -1.1070         -1.545  8
76   1.4940 -1.9080 0.5675          -0.9460                -1.343  7
78   1.7660 -1.9130         0.200900 -1.0330                -1.337  7
128  0.7861 -0.4951 1.2380  0.714600 -0.9454 -1.4850 -1.0820  -1.602 10
110  1.6760 -1.6440         0.452100 -1.0630         -0.6856  -1.325  8
96   1.0110 -1.1760 1.1170  0.322200 -0.9243 -1.1070         -1.532  9
80   1.3020 -1.9120 0.6784  0.321800 -0.9932                -1.330  8
112  1.2230 -1.6470 0.6633  0.568200 -1.0230         -0.6766  -1.319  9
10   2.0630 -2.4430                -1.5010                       5
12   1.6970 -2.4410 0.5967          -1.4490                       6
14   1.9470 -2.4440         0.283400 -1.5420                       6
```

46	1.8480	-2.1580	0.552800	-1.5670	-0.7144		7	
16	1.4590	-2.4410	0.7316	0.398800	-1.5040		7	
28	1.5800	-2.1610	0.7938	-1.4500	-0.4735		7	
48	1.3690	-2.1580	0.7162	0.664000	-1.5280	-0.7069	8	
32	1.3380	-2.1580	0.9328	0.404200	-1.5060	-0.4799	8	
64	1.1600	-1.6490	1.0250	0.737000	-1.5390	-0.7466	-0.8769	9
2	1.5450	-2.4360					4	
4	0.9799	-2.4340	0.9848				5	
6	1.6070	-2.4360	-0.138700				5	
20	0.8644	-2.1520	1.1800	-0.4776			6	
38	1.5140	-2.1810	0.092890	-0.6319			6	
8	0.9774	-2.4340	0.9861	0.003957			6	
40	0.8884	-2.1800	0.9785	0.234900	-0.6281		7	
24	0.8601	-2.1520	1.1830	0.006585	-0.4776		7	
56	0.6909	-1.6930	1.2700	0.297800	-0.7161	-0.7890	8	
9	1.1140		-1.4580				4	
11	0.6693		0.6805	-1.3590			5	
13	1.0650		0.113700	-1.4680			5	
15	0.4734		0.7955	0.305700	-1.3850		6	
1	0.5902						3	
3	-0.0531		1.1130				4	
5	0.6539		-0.140800				4	
7	-0.0800		1.1280	0.041650			5	

	logLik	AIC	delta	weight
74	-1119.920	2251.8	0.00	0.267
92	-1118.492	2253.0	1.14	0.151
76	-1119.629	2253.3	1.42	0.131
78	-1119.864	2253.7	1.89	0.104
128	-1117.072	2254.1	2.30	0.084
110	-1119.285	2254.6	2.73	0.068
96	-1118.344	2254.7	2.85	0.064
80	-1119.482	2255.0	3.12	0.056
112	-1118.917	2255.8	3.99	0.036
10	-1124.022	2258.0	6.20	0.012
12	-1123.726	2259.5	7.61	0.006
14	-1123.911	2259.8	7.98	0.005
46	-1123.244	2260.5	8.65	0.004
16	-1123.503	2261.0	9.16	0.003
28	-1123.513	2261.0	9.19	0.003
48	-1122.848	2261.7	9.86	0.002
32	-1123.283	2262.6	10.73	0.001
64	-1122.352	2262.7	10.86	0.001
2	-1128.191	2264.4	12.54	0.001

4 -1127.750 2265.5 13.66 0.000
6 -1128.173 2266.3 14.50 0.000
20 -1127.532 2267.1 15.22 0.000
38 -1127.644 2267.3 15.45 0.000
8 -1127.750 2267.5 15.66 0.000
40 -1127.227 2268.5 16.61 0.000
24 -1127.532 2269.1 17.22 0.000
56 -1126.768 2269.5 17.70 0.000
9 -1229.200 2466.4 214.56 0.000
11 -1228.720 2467.4 215.60 0.000
13 -1229.183 2468.4 216.53 0.000
15 -1228.596 2469.2 217.35 0.000
1 -1233.002 2472.0 220.16 0.000
3 -1232.332 2472.7 220.82 0.000
5 -1232.984 2474.0 222.13 0.000
7 -1232.331 2474.7 222.82 0.000
Models ranked by AIC(x)
Random terms (all models):
'1 | Sitef', '1 | Sitef:Hive'

Step 3:

```
> top.model <- get.models(model.set, subset=cumsum(weight)<=0.95)
Warning message:
In checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv,  :
  Model failed to converge with max|grad| = 0.00297695 (tol = 0.001, component 1)

> summary(top.model)
      Length Class     Mode
74  1       glmerMod S4
92  1       glmerMod S4
76  1       glmerMod S4
78  1       glmerMod S4
128 1       glmerMod S4
110 1       glmerMod S4
96  1       glmerMod S4
80  1       glmerMod S4
```

```
> mod.avg <- model.avg(top.model)
> summary(mod.avg)

CCall:
model.avg(object = top.model)

Component model call:
glmer(formula = Retour ~ <8 unique rhs>, data = res1, family =
    binomial, na.action = na.fail)

Component models:
        df   logLik     AIC delta weight
147      6 -1119.92 2251.84  0.00   0.29
12457    8 -1118.49 2252.98  1.14   0.16
1247     7 -1119.63 2253.26  1.42   0.14
1347     7 -1119.86 2253.73  1.89   0.11
1234567 10 -1117.07 2254.14  2.30   0.09
13467    8 -1119.28 2254.57  2.73   0.07
123457   9 -1118.34 2254.69  2.85   0.07
12347    8 -1119.48 2254.96  3.12   0.06

Term codes:
          Doser        LandLg10r            Tempr       VarroaLg10r
              1                2                3                4
 Doser:LandLg10r      Doser:Tempr Doser:VarroaLg10r
              5                6                7
```

Model-averaged coefficients:

(full average)

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)	
(Intercept)	1.4824	0.6032	0.6034	2.457	0.01402	*
Doser	-1.5903	0.6103	0.6105	2.605	0.00919	**
VarroaLg10r	-0.9703	0.4857	0.4859	1.997	0.04586	*
Doser:VarroaLg10r	-1.4103	0.4901	0.4904	2.876	0.00403	**
LandLg10r	0.4759	0.7532	0.7534	0.632	0.52763	
Doser:LandLg10r	-0.3924	0.7137	0.7139	0.550	0.58254	
Tempr	0.1628	0.4526	0.4528	0.360	0.71916	
Doser:Tempr	-0.1491	0.4378	0.4379	0.340	0.73355	

(conditional average)

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)	
(Intercept)	1.4824	0.6032	0.6034	2.457	0.01402	*
Doser	-1.5903	0.6103	0.6105	2.605	0.00919	**
VarroaLg10r	-0.9703	0.4857	0.4859	1.997	0.04586	*
Doser:VarroaLg10r	-1.4103	0.4901	0.4904	2.876	0.00403	**
LandLg10r	0.9051	0.8309	0.8313	1.089	0.27630	
Doser:LandLg10r	-1.2134	0.7609	0.7613	1.594	0.11099	
Tempr	0.4001	0.6391	0.6394	0.626	0.53148	
Doser:Tempr	-0.9047	0.6927	0.6930	1.305	0.19176	

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

2019

Retour => Homing
Doser => Dose
Tempr => temperature
VarroaLg10r => Varroa Log 10 tranformed
Land Lg10 => Landscape Log 10 transformed

Step 1:

>succes2<glmer(Retour~Doser*Tempr+Doser*VarroaLg10r+Doser*LandLg10r+(1|Sitef)+(1|Sitef:Hive), family=binomial, data=res1, na.action="na.fail")
Warning message:
In checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
Model failed to converge with max|grad| = 0.00139202 (tol = 0.001, component 1)
> summary(succes2)

Generalized linear mixed model fit by maximum likelihood (Laplace Approximation) [glmerMod]
Family: binomial (logit)
Formula: Retour ~ Doser * Tempr + Doser * VarroaLg10r + Doser * LandLg10r + (1 | Sitef) + (1 | Sitef:Hive)
Data: res1

AIC	BIC	logLik	deviance	df.resid
2194.9	2250.9	-1087.4	2174.9	1993

```
Scaled residuals:
    Min      1Q  Median      3Q     Max
-4.1763 -0.7779  0.3668  0.6667  2.6469

Random effects:
 Groups     Name        Variance Std.Dev.
 Sitef:Hive (Intercept) 0.4875   0.6982
 Sitef      (Intercept) 0.1224   0.3499
Number of obs: 2003, groups:  Sitef:Hive, 16; Sitef, 6

Fixed effects:
                 Estimate Std. Error z value Pr(>|z|)
(Intercept)       2.03952    0.55062   3.704 0.000212 ***
Doser            -2.97143    0.40634  -7.313 2.62e-13 ***
Tempr             0.33249    1.01862   0.326 0.744116
VarroaLg10r      -0.86152    0.71659  -1.202 0.229266
LandLg10r        -0.12341    0.73049  -0.169 0.865843
Doser:Tempr      -0.31852    0.78174  -0.407 0.683679
Doser:VarroaLg10r  1.08286    0.45878   2.360 0.018260 *
Doser:LandLg10r    0.08873    0.49750   0.178 0.858447
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of Fixed Effects:
            (Intr) Doser  Tempr  VrrL10 LndL10 Dsr:Tm D:VL10
Doser       -0.388
Tempr       -0.570  0.227
VarroaLg10r -0.424  0.210 -0.016
LandLg10r   -0.671  0.233  0.191  0.087
Doser:Tempr  0.227 -0.585 -0.346 -0.096 -0.070
Dsr:VrrLg10  0.233 -0.559 -0.111 -0.321 -0.060  0.220
Dsr:LndLg10  0.246 -0.648 -0.058 -0.070 -0.338  0.163  0.103
convergence code: 0
Model failed to converge with max|grad| = 0.00139202 (tol = 0.001, component 1)
```

<u>Step 2</u> :

```
> model.set <- dredge(succes2,rank="AIC")
Fixed term is "(Intercept)"
> model.set

Global model call: glmer(formula = Retour ~ Doser * Tempr + Doser * VarroaLg10r +
    Doser * LandLg10r + (1 | Sitef) + (1 | Sitef:Hive), data = res1,
    family = binomial, na.action = "na.fail")
---
```

Model selection table

	(Int)	Dsr	LL1	Tmp	VL1	Dsr:LL1	Dsr:Tmp	Dsr:VL1	df	logLik	AIC	delta	weight
74	2.0730	-3.015		-0.8679			1.120		6	-1087.583	2187.2	0.00	0.300
78	2.0200	-3.012		0.2136	-0.8746			1.116	7	-1087.557	2189.1	1.95	0.113
76	2.1200	-3.015	-0.11040		-0.8754			1.121	7	-1087.569	2189.1	1.97	0.112
2	1.7950	-2.624							4	-1090.798	2189.6	2.43	0.089
110	1.9750	-2.924		0.3662	-0.8470		-0.3427	1.073	8	-1087.457	2190.9	3.75	0.046
92	2.1490	-3.071	-0.17060		-0.8848	0.12220		1.128	8	-1087.539	2191.1	3.91	0.042
80	2.0610	-3.013	-0.08386	0.1904	-0.8796			1.117	8	-1087.549	2191.1	3.93	0.042
10	1.8830	-2.623		-0.3165					5	-1090.675			
6	1.7230	-2.623		0.2735					5	-1090.750			
4	1.8170	-2.624	-0.05385						5	-1090.794			
38	1.6540	-2.472		0.5775			-0.7072		6	-1090.308			
112	2.0150	-2.924	-0.07886	0.3437	-0.8518		-0.3413	1.074	9	-1087.451			
96	2.0900	-3.069	-0.14390	0.1898	-0.8891	0.12180		1.125	9	-1087.519			
14	1.8120	-2.621		0.2884	-0.3293				6	-1090.625			
12	1.9170	-2.623	-0.08091		-0.3218				6	-1090.668			
8	1.7310	-2.623	-0.01611	0.2693					6	-1090.749			
20	1.8190	-2.629	-0.05963			0.01210			6	-1090.794			
46	1.7400	-2.473		0.5903	-0.3139		-0.6974		7	-1090.195			
40	1.6590	-2.472	-0.01010	0.5748			-0.7071		7	-1090.308			
128	2.0390	-2.971	-0.12300	0.3333	-0.8606	0.08842	-0.3189	1.083	10	-1087.435			
16	1.8330	-2.621	-0.04222	0.2774	-0.3315				7	-1090.623			
28	1.9220	-2.632	-0.09066		-0.3227	0.02012			7	-1090.667			
24	1.7330	-2.628	-0.02166	0.2692		0.01158			7	-1090.749			
48	1.7570	-2.473	-0.03456	0.5810	-0.3156		-0.6969		8	-1090.194			
56	1.6460	-2.444	0.01815	0.5809		-0.05823	-0.7206		8	-1090.301			
32	1.8370	-2.630	-0.05185	0.2774	-0.3326	0.01982			8	-1090.622			
64	1.7450	-2.449	-0.01058	0.5858	-0.3128	-0.04922	-0.7084		9	-1090.189			
1	0.6428								3	-1224.796			
5	0.4927			0.5819					4	-1224.472			
9	0.7598			-0.4126					4	-1224.510			
3	0.7152		-0.17380						4	-1224.747			
13	0.6145			0.6012	-0.4354				5	-1224.184			
11	0.8508		-0.21320		-0.4233				5	-1224.439			
7	0.5417		-0.10450	0.5594					5	-1224.456			
15	0.6819		-0.14150	0.5699	-0.4402				6	-1224.157			

10 2191.4   4.18 0.037
6   2191.5   4.33 0.034
4   2191.6   4.42 0.033
38 2192.6   5.45 0.020
112 2192.9   5.74 0.017
96 2193.0   5.87 0.016
14 2193.2   6.08 0.014
12 2193.3   6.17 0.014
8   2193.5   6.33 0.013
20 2193.6   6.42 0.012
46 2194.4   7.22 0.008
40 2194.6   7.45 0.007
128 2194.9   7.70 0.006
16 2195.2   8.08 0.005
28 2195.3   8.17 0.005
24 2195.5   8.33 0.005
48 2196.4   9.22 0.003
56 2196.6   9.43 0.003
32 2197.2 10.08 0.002
64 2198.4 11.21 0.001
1   2455.6 268.43 0.000
5   2456.9 269.78 0.000
9   2457.0 269.85 0.000
3   2457.5 270.33 0.000
13 2458.4 271.20 0.000
11 2458.9 271.71 0.000
7   2458.9 271.74 0.000
15 2460.3 273.15 0.000
Models ranked by AIC(x)
Random terms (all models):
'1 | Sitef', '1 | Sitef:Hive'

Step 3 :

```
> top.model <- get.models(model.set, subset=cumsum(weight)<=0.95)
> summary(top.model)
```

```
   Length Class    Mode
74  1      glmerMod S4
78  1      glmerMod S4
76  1      glmerMod S4
2   1      glmerMod S4
110 1      glmerMod S4
92  1      glmerMod S4
80  1      glmerMod S4
10  1      glmerMod S4
6   1      glmerMod S4
4   1      glmerMod S4
38  1      glmerMod S4
112 1      glmerMod S4
96  1      glmerMod S4
14  1      glmerMod S4
12  1      glmerMod S4
8   1      glmerMod S4

> mod.avg <- model.avg(top.model)
> summary(mod.avg)

Call:
model.avg(object = top.model)

Component model call:
glmer(formula = Retour ~ <16 unique rhs>, data = res1, family =
    binomial, na.action = na.fail)

Component models:
      df   logLik     AIC delta weight
147    6 -1087.58 2187.17  0.00   0.32
1347   7 -1087.56 2189.11  1.95   0.12
1247   7 -1087.57 2189.14  1.97   0.12
1      4 -1090.80 2189.60  2.43   0.09
13467  8 -1087.46 2190.91  3.75   0.05
12457  8 -1087.54 2191.08  3.91   0.05
12347  8 -1087.55 2191.10  3.93   0.04
14     5 -1090.68 2191.35  4.18   0.04
13     5 -1090.75 2191.50  4.33   0.04
12     5 -1090.79 2191.59  4.42   0.03
136    6 -1090.31 2192.62  5.45   0.02
123467 9 -1087.45 2192.90  5.74   0.02
123457 9 -1087.52 2193.04  5.87   0.02
134    6 -1090.62 2193.25  6.08   0.02
```

```
124     6 -1090.67 2193.34  6.17   0.01
123     6 -1090.75 2193.50  6.33   0.01

Term codes:
          Doser        LandLg10r           Tempr       VarroaLg10r
              1                2               3                4
 Doser:LandLg10r      Doser:Tempr Doser:VarroaLg10r
              5                6               7

Model-averaged coefficients:
(full average)
                  Estimate Std. Error Adjusted SE z value Pr(>|z|)
(Intercept)       1.994445   0.394771    0.394984   5.049    4e-07 ***
Doser            -2.903423   0.297535    0.297649   9.755   <2e-16 ***
VarroaLg10r      -0.658406   0.718615    0.718943   0.916    0.360
Doser:VarroaLg10r 0.815219   0.625921    0.626064   1.302    0.193
Tempr             0.091532   0.564259    0.564580   0.162    0.871
LandLg10r        -0.031670   0.375427    0.375650   0.084    0.933
Doser:Tempr      -0.037679   0.262054    0.262174   0.144    0.886
Doser:LandLg10r   0.007564   0.126024    0.126097   0.060    0.952

(conditional average)
                  Estimate Std. Error Adjusted SE z value Pr(>|z|)
(Intercept)        1.9944     0.3948      0.3950   5.049    4e-07 ***
Doser             -2.9034     0.2975      0.2976   9.755   <2e-16 ***
VarroaLg10r       -0.8231     0.7142      0.7146   1.152   0.2493
Doser:VarroaLg10r  1.1155     0.4485      0.4487   2.486   0.0129 *
Tempr              0.2735     0.9495      0.9501   0.288   0.7735
LandLg10r         -0.1034     0.6729      0.6733   0.154   0.8779
Doser:Tempr       -0.4290     0.7836      0.7841   0.547   0.5842
Doser:LandLg10r    0.1221     0.4923      0.4926   0.248   0.8042
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```